STUDIES OF THE INTERACTIONS OF CCA AND ACA PRESERVATIVE TREATED WOOD WITH SOIL.

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This thesis is presented to the Council for National Academic Awards in partial fulfilment of the requirements for the award of the degree of Doctor of Philosophy.

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TO MY PARENTS

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ABSTRACT

Chemical and microbiological changes at the interface between soil, and wood treated with CCA or ACA wood preservatives were investigated using a series of leaching and soil burial studies. The softwoods Scots pine (*Pinus sylvestris*, L.), Sitka spruce (*Picea sitchensis*, Carr) and the hardwood lime (*Tilia vulgaris*, Hayne) were used exclusively.

Copper losses from both types of preservative treated wood were negligible, though adjacent soil copper concentrations significantly increased. These copper accumulations were associated with a reduction in dehydrogenase activity around the preservative treated material compared with levels around the untreated blocks, though activity around the treated wood was rarely less than background levels. Relatively large arsenic concentrations accumulated around the most heavily ACA-treated blocks, and were associated with a further reduction in activity of the soil microflora. The wood species also affected the microbial activity in adjacent soil; activity around all lime blocks was generally greater than microbial activity around the softwoods.

Treatment of wood with ammonia or ACA solutions increased the wood nitrogen contents. Some of this nitrogen was readily water soluble, though its rapid diffusion into adjacent soil had no effect on microbial activity in this area. Water insoluble nitrogen was also retained within these blocks; this was shown to increase the rate of microbial colonisation and decay of the wood and was also associated with an increased toxic value of copper.

Microbial activity was measured in all decaying wood blocks. This activity was influenced by the wood species, and treatment, as were the microbial colonisation and decay rates. The experimental conditions employed were designed to promote soft rot, rather than other forms of wood decay. Activity was greater in the outer wood surface of the buried blocks than in the inner wood, reflecting the surface nature of soft rot decay.

Pre-burial leaching reduced the subsequent moisture uptake and increased the durability of CCA-treated wood during soil burial, though untreated wood was unaffected. However, similar rates of microbial decay of untreated wood blocks occurred over a range of different wood moisture contents.

The implications of the findings on the relative performances of untreated, ammonia, CCA and ACA-treated wood in soil contact are discussed.

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CHAPTER 1

INTRODUCTION

INTRODUCTION

1.1 General introduction.

Wood is the one structural material used extensively by man that can be regarded as a renewable resource (Levy and Dickinson, 1981). Wood can be partially or completely destroyed by fire, chemical or mechanical agencies, or by biodeterioration (Levy and Dickinson, 1981 *op cit*). Organisms cause three major kinds of damage to wood and these are conventionally referred to in terms of the type of organism responsible, namely marine borer damage, insect damage and microbiological degradation (Scheffer, 1973). Of these three, microorganisms are responsible for the largest wood losses (Scheffer, 1973 *op cit*). Microbiological degradation can be prevented if wood is maintained in a dry state, i.e. with a moisture content of less than 20% (Scheffer, 1973 *op cit*). Where wood is employed in conditions in which its moisture content is likely to be greater than 20%, or in circumstances in which there is considered to be a decay hazard, the use of preservatives is necessary to avoid such degradation. In this context timber preservation refers to the improvement of wood's natural durability by treatment with chemicals that are toxic to insects, fungi and marine borers (Wilkinson, 1979).

Soil is the most aggressive environment to which preserved wood may commonly be exposed (Hilditch, 1978; King *et al*, 1981a), since it contains moisture and additional nutrients, as well as an inoculum of microorganisms capable of degrading organic material. Both untreated and preservative treated wood in ground contact exert an influence on the surrounding soil, and *vice versa*. Work reported in this thesis is concerned with the chemical and microbiological changes that occur at the wood/soil interface when untreated and preservative treated wood is buried in soil.

A number of factors can influence the colonisation and degradation of wood by soil microorganisms, such as the wood species, the moisture and nitrogen contents of the wood, and the presence of a preservative. The efficacy of some wood preservatives, for example copper chrome arsenate (CCA), is affected by the structural and chemical composition of the wood. Thus a knowledge of wood structure and chemical make-up is important in understanding the degradation of wood in soil and the performance of some preservatives.

1.2 Wood: structure and chemical composition.

1.2.1 Introduction.

Timbers divide naturally into two distinct groups: the softwoods and the hardwoods (Levy and Dickinson, 1981). These terms refer to different species of trees and not to the hardness and softness of the wood (Gray and Parham, 1982). The softwoods, or gymnosperms, include commercially important species such as pine, spruce, Douglas fir and redwood. Ash, beech, walnut and oak are examples of commercially important hardwoods, or angiosperms (Levy and Dickinson, 1981 *op cit*; Gray and Parham, 1982 *op cit*).

One of the most characteristic features of both softwood and hardwood timbers is the presence of differently coloured zones of wood (Wilkinson, 1979). The outer, or sapwood, zone is usually a creamy colour, while the inner area, or heartwood, is frequently darker (Wilcox, 1973), though the difference is not always so distinct (Findlay, 1985a). The sapwood can be defined as the portion of the wood in the living tree that contains living cells and reserve materials, such as starch (Wilkinson, 1979 *op cit*). The heartwood can be defined as the layers of wood in the growing tree which have

ceased to contain living cells, and in which reserve materials, such as starch have been removed or converted into heartwood substances (Wilkinson, 1979 *op cit*).

Although the sapwood of most species is perishable, the range of natural durability of the heartwood zones varies from species to species, and is frequently greater than that of the sapwood (Wilkinson, 1979 *op cit*; Levy and Dickinson, 1981). The greater durability of the heartwood zone has been attributed to the presence of toxic extractives in the cell walls, such as tannins, and to the lack of readily available nutrients (Wilkinson, 1979 *op cit*; Levy and Dickinson, 1981 *op cit*). Furthermore, in the preservative treatment of round poles it is only considered essential to achieve complete penetration of the sapwood band (Findlay, 1985a), since this is the area which is directly in contact with the environment, and is also the most perishable zone. In the work reported here only sapwood was employed, therefore, only the sapwood area of the softwoods and hardwoods will be discussed further.

The microscopic structure and the chemical composition of softwoods and hardwoods are very different. These differences are thought to account for the greater soft rot decay susceptibility of the hardwoods, even when preservative treated (Greaves, 1972, 1974; Dickinson, 1974; Hulme and Butcher, 1977c; Butcher and Nilsson, 1982), therefore, an understanding of the structural and chemical differences between the two wood types is essential in interpreting results obtained in the current work. Detailed treatises on both wood structure and chemical composition are available (Jane, 1970; Wenzl, 1970), so only brief reviews of these areas will be presented.

1.2.2 Structural composition of softwoods and hardwoods.

Both wood types are composed of two interwoven systems of cells, one from root to crown (longitudinal), and the other from bark to pith (radial) (Wilkinson, 1979). The softwoods are simpler and more uniform in structure than the hardwoods, being constituted principally of vertically oriented cells called tracheids (Jane, 1970 *op cit*; Levy and Dickinson, 1981). In the softwoods the tracheids make up 90% of the total cell volume, with horizontally oriented ray cells and resin ducts running either horizontally or vertically constituting the remainder (Gray and Parham, 1982). In the living tree the tracheids act as both the means of support and of fluid conduction, while the ray cells contain stored food reserves, such as starch (Wilkinson, 1979 *op cit*; Gray and Parham, 1982 *op cit*). The resin ducts, although a constant feature in species of pine, larch, spruce and Douglas fir, are only occasionally present in other softwoods (Gray and Parham, 1982 *op cit*).

The tracheids are hollow, square to rectangular in cross-section, and have closed, tapered, overlapping ends (Gray and Parham, 1982 *op cit*). The flow of liquid from one tracheid to another is achieved through small openings in the tracheid wall, called pits (Wilkinson, 1979 *op cit*; Gray and Parham, 1982 *op cit*). Across adjoining pits is a specialised pit membrane, which can be moved as the liquid (sap) pressure varies in adjacent fibres, allowing some control of liquid flow (see figure 1.1; Gray and Parham, 1982 *op cit*).

The hardwoods are structurally more complex than the softwoods (Jane, 1970 *op cit*) and their cells show a greater variation in size and shape (Wilkinson, 1979 *op cit*), as well as a less ordered configuration (Gray and Parham, 1982 *op cit*). Support for the tree is

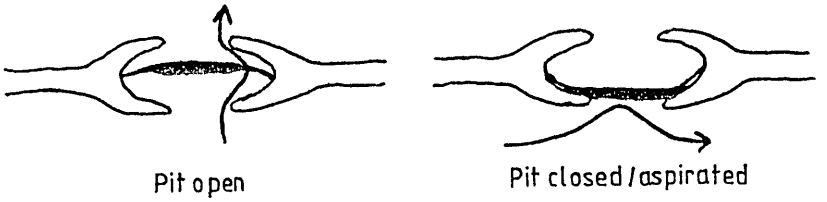


Figure 1.1 Simplified structure of a softwood pit (from Wilkinson, 1979).

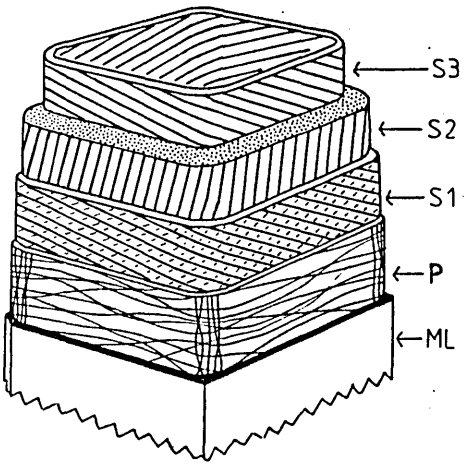


Figure 1.2 Diagram of part of a fibre or tracheid showing the cell wall layers and the usual orientation of the microfibrils in the various layers of the wall (Jane, 1970).

provided mainly by fibres, the principal cell type in hardwoods (Wilkinson, 1979 *op cit*). The hardwood fibres are very like the softwood tracheids in structure, but are much shorter with relatively thick walls and considerably fewer pits (Gray and Parham, 1982 *op cit*). Some conduction of liquids may be achieved via the fibres, though the majority of conduction takes place in the vessel elements, which connect end-to-end to form long pipe-like structures called vessels (Wilkinson, 1979 *op cit*). The vessel elements are the largest type of wood cells (Wilkinson, 1979 *op cit*), and in some wood species the diameter is such that individual vessels are visible to the naked eye (Gray and Parham, 1982 *op cit*). The vessel elements are either open ended (simple perforation plates), or have an open grill-like structure (scalariform perforation plates) (Levy and Dickinson, 1981). In either case the hardwood vessels provide an open pathway or network for the passage of liquids or organisms (Levy and Dickinson, 1981 *op cit*). Hardwood storage tissue consists of parenchyma cells in both the longitudinal (longitudinal parenchyma) and horizontal (ray parenchyma) orientations (Wilkinson, 1979 *op cit*).

1.2.3 Refractory wood species.

In most wood species the sapwood can be easily impregnated with preservatives (Wilkinson, 1979 *op cit*). This is not unexpected, since in the growing tree the sapwood contains the cells that conduct water and minerals from the roots to the leaves (Wilkinson, 1979 *op cit*; Findlay, 1985a). However, the sapwood of eastern hemlock (*Tsuga conadensis*) and many firs (*Abies* species) and spruces (*Picea* species) are penetrated by preservatives to only a limited extent (Wilkinson, 1979 *op cit*), and are therefore referred to as difficult-to-treat or refractory wood species. The refractory nature of these wood species

is due to the pit membranes of the early wood tracheids being drawn to one side of the pit and becoming tightly bound to the tracheid wall during drying of the wood (aspirated pits, see figure 1.1) (Wilkinson, 1979 *op cit*; Gray and Parham, 1982). Cells with aspirated pits are unable to conduct liquids (Wilkinson, 1979 *op cit*) reducing the natural permeability of the wood (Gray and Parham, 1982 *op cit*). Various methods have been used in an attempt to improve the penetration of preservatives into refractory wood species, including incising, wet storage, steaming, differential diffusion techniques (Johansson and Nordman-Edberg, 1987) and the use of ammoniacal wood preservatives, such as ACA (Rak and Clarke, 1974).

1.2.4 Chemical composition and cell wall structure of wood.

Wood is composed of three polymeric materials: cellulose, the hemicelluloses and lignin (Kirk, 1973). Other substances, such as nitrogenous materials, pectin, starch, low molecular weight sugars and minerals are also present, along with varying amounts of extraneous materials (lignans, terpenes, polyphenols, etc.) (Kirk, 1973 *op cit*).

The cellulose content of hardwoods and softwoods is similar (Cote, 1977), at 40-50% of the total dry weight of wood for the majority of temperate-zone woods (Kirk, 1973 *op cit*). However, softwoods contain appreciably more lignin than hardwoods (Cote, 1977 *op cit*; Findlay, 1985a), at 25-34% and 17-24% respectively (Kirk, 1973 *op cit*). The composition of lignin in the two wood types also varies; in softwood lignins guaiacyl is the basic building unit, but the primary building unit for hardwood lignins is syringyl (Thomas, 1977). Typically softwood lignin contains ten times more guaiacyl than syringyl, whereas in hardwoods the ratio is 1:1 (Thomas, 1977 *op*

cit). Since the guaiacyl unit has a greater number of potential reactive sites, a higher degree of crosslinking exists in the softwood lignins, thus, the hardwood lignins are more easily degraded (Thomas, 1977 *op cit*). Hemicellulose comprises the majority of the remainder of the wood substance for both wood types (Kirk, 1973 *op cit*), with the extractives constituting only a small percentage (2-5%) (Thomas, 1977 *op cit*). The hemicellulose type and quantity of the hardwoods and softwoods also varies (Kirk, 1973 *op cit*; Cote, 1977 *op cit*; Levy and Dickinson, 1981).

Although some variability exists, the internal cell wall structure described below typifies that found in most mature woody plant cells. Wood cell walls of both hardwoods and softwoods are complex layered structures (Wilkinson, 1979). Figure 1.2 shows a schematic diagram of the wood cell wall. Each cell has a primary wall and a secondary wall, which consists of three layers (known as S1, S2 and S3) (figure 1.2; Jane, 1970; Wilkinson, 1979 *op cit*). Adjacent cells are joined by a thin layer of intercellular material called the middle lamella. Both the middle lamella and the primary wall of mature wood cells are heavily lignified (Kirk, 1973 *op cit*).

The cellulose molecules of the wood cell walls are organised into strands known as microfibrils (Kirk, 1973 *op cit*). The microfibril orientation within the wood cell wall varies with the wall layer (figure 1.2; Thomas, 1977; Gray and Parham, 1982). It is considered (Kirk, 1973 *op cit*) that the hemicelluloses are present in an amorphous state in the cell walls and, together with the lignin, surround the cellulose microfibrils as a matrix (Gray and Parham, 1982 *op cit*). The nature of the association between the lignin and the hemicellulose is not clear, though the hemicelluloses and the lignin are thought to form an interpenetrating polymer complex, covalently bonded to one another (Kirk, 1973 *op cit*).

The S2 layer is the thickest layer of the wood cell wall (Thomas, 1977 *op cit*), comprising about 70-75% of the total wall volume (Gray and Parham, 1982 *op cit*) and is the dominant cell wall layer, since its size and microfibril orientation (figure 1.2) give it great strength (Gray and Parham, 1982 *op cit*). The S1 and S3 layers are thinner than the middle layer, with the S1 layer being slightly thicker than the S3 (Gray and Parham, 1982 *op cit*). The concentration of lignin in all three secondary cell wall layers is considerably lower than in either the primary wall or the middle lamella. However, due to the very large volume of the S2 layer, over half the total lignin is found in this layer (Thomas, 1977 *op cit*).

The majority of wood constituents are utilisable by microorganisms of some kind (Scheffer, 1973), though some components, such as lignin, require specialised fungi, such as the white rot basidiomycete fungi, to degrade them.

1.3 Wood decay.

1.3.1 Microbial colonisation and decay of wood in soil contact.

Wood decay can be defined as "the decomposition of wood substance caused by action of wood destroying fungi, resulting in softening, loss of strength and weight, and often changes of texture and colour" (ASTM D9-87: 1987). A number of microbial groups are capable of colonising untreated and preservative treated wood, though they do not always cause decay (Clubbe and Levy, 1982). Ecological studies of untreated sapwood in soil contact (Corbett and Levy, 1963; Butcher, 1968; Banerjee and Levy, 1971; Clubbe and Levy, 1982 *op cit*) have shown the succession of microbial groups which colonise wood over a time period generally approximates to:

bacteria - primary moulds - stainers - soft rot fungi - basidiomycetes and secondary moulds.

The soft rot fungi generally tolerate higher concentrations of toxic chemicals than basidiomycetes (Duncan, 1960; Bravery, 1975) and are considered responsible for most of the ultimate decay of the treated zone of timber (Smith, 1969a). Therefore, it is not surprising that treating wood with CCA generally excluded the basidiomycetes from the ecological succession determined (Clubbe and Levy, 1982 *op cit*). The succession of microbial groups for the CCA-treated wood was from bacteria to soft rot fungi, as it was for the untreated wood, with the soft rot fungi being the climax group (Clubbe and Levy, 1982 *op cit*). CCA increased the time taken to progress through the succession sequence and the number of individual species involved in each microbial group was reduced (Butcher, 1968 *op cit*).

Banerjee and Levy (1971 *op cit*) only detected basidiomycetes in the inner areas (5mm and deeper) of the ground line of untreated

birch and Scots pine posts. At the surface of the poles a greater variety of fungal species were isolated than in the other areas investigated, and the succession of microflora observed was very similar to that described for CCA-treated material. The exclusion of basidiomycetes from the surface layers of the poles was attributed to the intense fungal activity in this area, which was presumed to provide a severe restriction to colonisation by the higher fungi (Banerjee and Levy, 1971 *op cit*). Therefore at the wood/soil interface of both untreated and preservative treated wood the succession of microbial groups is likely to be:

bacteria - primary moulds - stainers - soft rot fungi.

Clubbe and Levy (1982) regarded the bacteria, primary moulds and stainers as early passive colonisers. They considered them to be incapable of decaying the wood directly, therefore being reliant on simple sources of nutrients, either already present in the wood, or carried there from the soil surrounding the stake. However, while these early colonising microbes do not generally cause substantial degradation of the wood structure, some are capable of causing a degree of physical change in the wood.

Bacteria have been shown to increase the permeability of softwoods (Levy, 1975), and this has been attributed to the partial, or total destruction of the pit membranes by the bacteria. The increased permeability of softwoods after bacterial colonisation has been employed in efforts to increase the permeability of refractory wood species in the procedure known as ponding (Wilkinson, 1979). Opening up the pit membranes of wood in soil contact can result in less anaerobic conditions inside the wood, and may also produce open pathways for colonising fungi (Levy and Dickinson, 1981). Bacteria can produce significant decay of the wood cell wall, primarily the S2 layer (Nilsson and Daniel, 1983; Nilsson and Singh, 1984; Singh and

Butcher, 1985). This bacterial decay has generally been observed in wood which is resistant to other forms of decay, either because of preservative treatment or the chemical composition of the wood.

The primary moulds appear unable to degrade the wood cell wall, and are likely to be reliant on sugars or simple carbohydrates present in wood (Levy and Dickinson, 1981 *op cit*). The stainers tend to colonise the ray parenchyma cells of the sapwood, utilising the cell contents and stored food reserves as a nutrient source (Levy and Dickinson, 1981 *op cit*). However, some species within this group are considered capable of causing soft rot (King and Oxley, 1975; Levy and Dickinson, 1981).

Potential early colonisers of wood may have an advantage over other colonisers if they have the ability to produce 'antibiotic' substances which are both bacteriostatic/cidal and fungicidal (Kelly *et al*, 1980). Three early wood colonisers (*Trichoderma viride*, *Gliocladium virens* and *Paecilomyces marquandi*) were found to produce antibiotic substances in laboratory media which inhibited bacterial growth (Kelly *et al*, 1980 *op cit*). Bruce and King (1983) demonstrated that infection of wood blocks with strains of *Trichoderma* and *Scytalidium* for periods of up to 3 months prior to the exposure of the blocks to *Lentinus lepideus* conferred residual protection against wood decay by *L.lepideus*, even when the control organisms were killed and the blocks thoroughly leached. However, microbial interactions on laboratory media may be very different to the interactions observed in the natural habitat (Kaarik, 1968).

The first wood decay fungi to appear in the microbial succession in wood in soil contact are the soft rots (Clubbe and Levy, 1982). Savory (1954a) coined the term "soft rot" for the decay caused by cellulose-destroying microfungi, after observing surface softening of the wood as a result of decay by these fungi. Currently all types of

wood decay caused by microfungi, such as ascomycetes and Fungi Imperfecti (Levy and Dickinson, 1981 *op cit*), are called soft rot, regardless of whether the wood surfaces have been softened or not (Nilsson, 1976).

Timber decayed by soft rot fungi is darkened and softened on the surface while wet (Savory, 1954b; Duncan, 1960, Wilkinson, 1979). On drying the decayed surface hardens and cross-cracking is produced (Savory, 1954b *op cit* and Wilkinson, 1979 *op cit*). However, the depth of fungal penetration is limited, apparently by the oxygen supply (Savory, 1954a and 1955). Thus, soft rot is particularly important on timbers with large surface area to volume ratios (Savory, 1955 *op cit*).

Hyphae of the soft rot fungi are typically observed running longitudinally within the S2 layer of the secondary wall of the wood (Savory, 1954a, 1954b *op cit*; Duncan, 1960 *op cit*), where they form chains of cavities (Corbett, 1965). The soft rot attack can be regarded (Nilsson, 1982) as a series of consecutive steps as follows:

1. colonisation of the wood substrate;
2. hyphal penetration of the wood fibre walls;
3. formation of T-branches (or L-branches).

   In this way the cavity-forming hyphae orient themselves along the cellulose microfibrils in the S2 layer.
4. formation of cavities around the oriented hyphae. A widening of the cavities occurs as a result of enzymic activity.

While soft rot fungi are known to be cellulose attacking species (Savory, 1954a *op cit*), there is some evidence that they can decompose lignin (Savory, 1955; Savory and Pinion, 1958). This generally occurs in the later stages of decay, when associated carbohydrates are also attacked.

Generally softwoods are more resistant to soft rot than hardwoods

(Savory, 1954a *op cit*; Duncan, 1960 *op cit*; Smith, 1969a; Behr, 1973; Hulme and Butcher, 1977c) and under in-service conditions soft rot appears to be more prevalent in hardwoods than softwoods (Duncan, 1960 *op cit*). Toxic values for CCA and ACA (the concentration range of the preservative which just inhibits decay and that which just permits it, Bravery, 1975) are much higher for hardwoods than for softwoods (Hulme and Butcher, 1977b; 1977c).

Usually the majority of microorganisms present in natural soil are not actively metabolising. Viable spores of fungi do not usually germinate in natural soils, but lie dormant. This phenomenon is termed fungistasis (Griffin *et al*, 1975). Fungistasis is attributed either to nutrient deprivation (Lynch, 1982), or to a fungistatic factor. Fungistasis can be temporarily relieved by nutrient additions (Dobbs and Hinson, 1953; Griffin *et al*, 1975 *op cit*). Similarly, bacteriostasis also occurs in soils (Brown, 1973; Davis, 1976). Before wood blocks can suffer any form of microbial attack, spore germination must first be induced.

Murphy (1982) observed that soon after the emplacement of wood in soil there was an increase in the occurrence of "opportunistic" fungi in soil around the wood. These fungi were able to colonise wood, but had no wood degrading ability (Murphy, 1982). Smith (1980) suggested that, when untreated specimens are put into soil, traces of soluble nutrients from wood could diffuse to nearby propagules, annul fungistasis and stimulate germination. However, Mowe (1983) demonstrated that volatile emissions from dried wood in the vicinity of fungal inocula stimulated hyphal growth towards the wood, indicating that nutrient diffusion may not be necessary for stasis release. It was suggested (Mowe, 1983 *op cit*) that the wood volatiles initiated both fungal spore germination and oriented hyphal growth towards the wood. Mowe (1983 *op cit*) also demonstrated that certain

motile bacteria exhibited a positive chemotactic response to water soluble wood extracts, so that bacterial colonisation of wood could be promoted by the movement of soluble materials into the surrounding soil.

Following the increased isolation of "opportunistic" fungi, Murphy (1982 *op cit*) isolated increasing numbers of organisms which have the ability to colonise the wood in depth and utilise it. He designated these two phases the "initial phase" and the establishment phase. Fungal species isolated from adjacent soil were also isolated from the wood (Murphy, 1982 *op cit*), confirming the observations of King *et al* (1980a), that the soft rot phenomenon is one in which the biotic connection is retained with soil by invading organisms.

Smith (1980) noted that the time taken for decay to begin in wood increased exponentially with the preservative concentration, as did the period up to complete failure of the specimen (as determined by strength testing). He suggested that when treated specimens are placed in soil, sub-lethal amounts of preservative could diffuse into soil and delay or prevent germination, as well as delaying colonisation.

Some accumulation of preservative metals has been measured in soil adjacent to preservative treated wood (De Groot *et al*, 1979; Murphy, 1982 *op cit*). However, Murphy (1982 *op cit*) did not observe any general inhibition of fungal germination in soil around CCA-treated stakes. Instead the "initial phase" observed in adjacent soil was very similar to that previously described for the untreated wood stakes. However, during the "establishment phase" the frequency of isolation of copper-tolerant fungi from soil adjacent to preservative treated stakes remained high, while it decreased in soil surrounding untreated material. Yamamoto *et al* (1985) also demonstrated an increase in the isolation of copper tolerant fungi in

soil artificially polluted with copper (up to 1600ug g^{-1} dry soil). Differences in fungal species colonising untreated and CCA-treated wood have been noted: *Phialophora* species are generally the predominant soft rot fungi isolated from CCA-treated wood, and the adjacent soil (Nilsson and Henningsson, 1978; Murphy, 1982 *op cit*), though this is not the case for the untreated wood.

Clubbe and Levy (1982) observed bacteria, primary moulds, stainers and soft rot fungi colonising stakes of birch and pine, treated with CCA at relatively low retentions and placed in soil contact. The soft rot fungi colonising the treated pine stakes had not caused any decay by the conclusion of the study. These fungi were presumed to have had either an external, soil based nutrient supply, or been saprophytic on the earlier colonists (Clubbe and Levy, 1982 *op cit*).

Bravery (1968b) observed that increasing the incubation period of a soft rot test produced a corresponding and marked increase in weight loss recorded at any given treating concentration, a factor which becomes extremely important when determining toxic values. He suggested that this was probably due to the retardation of the active colonisation by the fungi at increased concentrations of preservative, which is then gradually overcome after a period of "physiological adjustment" of the fungal population, rather than to the gradual leaching of the preservative. The apparent selection of copper tolerant fungi in soil adjacent to CCA-treated wood in soil contact observed by Murphy (1982 *op cit*) could be the "physiological adjustment" of the fungal population proposed by Bravery (1968b, *op cit*).

1.3.2 The moisture content of wood in relation to wood decay.

The moisture content of wood has a significant effect on the susceptibility of the wood to microbial decay and the type of decay that takes place. The decay fungi can only cause serious damage to the wood when the moisture content is greater than the fibre saturation point (Scheffer, 1973). Therefore, a widely used and effective means of protecting wood from microbial degradation is to dry it soon after it comes out of the tree and then maintain it in this dry state (Scheffer, 1973 *op cit*). The fibre saturation point is the precise moisture content at which wood has lost all its "free water", but none of its "bound water". "Free water" is defined as water not bonded to the wood, which can be removed by evaporation and "bound water" is water which is chemically or physically trapped within the wood and can only be removed by extreme methods, such as oven-drying, (Wilkinson, 1979). The fibre saturation point of most timbers occurs at moisture contents of between 24 and 30% (Wilkinson, *op cit*). Wood has a capacity to absorb moisture either as a liquid or a vapour (Oxley *et al*, 1976; Morgan, 1986). Therefore, when wood is used in environments where water is freely available, such as marine or soil situations, preservation is generally essential.

Soft rot fungi have been reported to be capable of attacking wood which is too wet or too dry for Basidiomycete decay (Savory, 1955, Behr, 1973). Becker and Kaune (1966) reported that the lower limit of wood moisture for decomposition of pine and beech by soft rot fungi was 30-35%. The same authors reported an upper moisture content limit of 60-80% for the decay of pine by soft rot fungi, though decay has been measured at moisture contents in excess of 100% (Gray, 1986). Morton and Eggins (1976) demonstrated that

thermophilic, cellulolytic microfungi were capable of growing in timbers with moisture contents of less than 20% when wood samples were maintained at temperatures greater than 30°C.

CCA-treated wood blocks have been demonstrated to have lower moisture uptakes on emplacement in soil compared with that attained by untreated blocks (Baines, 1982; Murphy, 1982; Gersonde and Kerner, 1984; Pizzi and Conradie, 1986; Gray, 1986). The durability of CCA-treated wood has been associated with this increased hydrophobic character (Pizzi and Conradie, 1986, *op cit*).

There is less information on moisture uptake by aqueous ammonia or ACA-treated wood on emplacement in soil compared with either untreated or CCA-treated wood. The increased hydrophobic nature of CCA-treated wood has been attributed to the chromium (VI) component of the preservative (Pizzi and Conradie, 1986, *op cit*). Thus, a reduction in moisture uptake by ammonia or ACA-treated wood is unlikely. However, when the carbonate ion is present in the ACA formulation, water repellency has been shown to be imparted to the wood (Rak and Clarke, 1974). Copper carbonate was employed in the ACA formulation used in the work reported here.

1.3.3 Wood decay and wood nitrogen content.

The nitrogen content of wood is low and this tends to limit the utilisation of the substrate by microorganisms (Findlay, 1934; Levi and Cowling, 1969; Levy, 1973). However, where microbial colonisation and decay of wood occurs, increases in the wood nitrogen content have been measured (Mowe, 1983). It has been shown (Waite and King, 1980) that the nitrogen increases measured are primarily due to

microorganisms. Therefore monitoring the nitrogen content of wood in soil contact provides a means by which the microbial colonisation of the wood can be followed.

Wood is considered to be a very deficient substrate for colonisation and decay by fungi (Findlay, 1934; Levi and Cowling, 1969; Levy, 1973), due to its low nitrogen content in comparison to other plant tissues (Cowling and Merrill, 1966). Only a small percentage of sapwood nitrogen is soluble (Merrill and Cowling, 1966; Baker, Laidlaw and Smith, 1970) therefore, the bulk of the nitrogen present in wood is not immediately available to colonising microorganisms (King and Oxley, 1975). Since much of this soluble nitrogen is redistributed to evaporative surfaces during drying of green wood (King, Oxley and Long, 1974), the soluble nitrogen content of sub-surface samples will be reduced. This was demonstrated by Nayagam (1987) for samples of Scots pine, Sitka spruce and lime, all of which had been obtained from the same source as those utilised in the current work. Sub-surface samples were used in all experiments carried out during the work reported here.

At the carbon:nitrogen ratios that normally occur in wood, soft rot fungi do not produce cellulolytic enzymes (Levi and Cowling, 1969), though when nitrogen is added to the wood the rate of production of these enzymes and the rate of decay is increased in pure culture tests (Savory, 1954a, 1954b; Levi and Cowling, 1969 *op cit*). Levi and Cowling (1969 *op cit*) found that a carbon:nitrogen ratio of less than 200:1 was required to produce cellulase activity in the majority of soft rot fungi tested, and Waite and King (1979) observed that a minimum nitrogen value of about 0.2%w/w (carbon:nitrogen ratio of approximately 140:1), was required before the cellulase system of the microfungi would become optimally active.

Soft rot fungi do decay wood, despite its meagre nitrogen supply,

therefore the nitrogen content of wood must be increased to its required minimum value by some means.

During drying of green wood soluble nitrogen (King *et al*, 1974) and simple sugars (Long, 1978) migrate to evaporative surfaces, where they are deposited in concentrations up to five times and ten times respectively those found in sub-surface wood. The presence of these redistributed soluble nutrients (RSN) in untreated wood has been found to increase the depth of soft rot decay (King *et al*, 1976) and enhance the initial decay rate (Waite and King, 1979) when compared to wood without RSN. Presence of RSN was also shown to increase the toxic values of CCA (King *et al*, 1981b)

Baines and Levy (1979) showed that when one end of a small wooden stake was immersed in water, and the other left above the surface of the water, there was a movement of water through the wood to the exposed end. With evaporation of water from the exposed end of the wood, there was a continuing flow of water through the stake. It was suggested that this water flow, known as "wick action", may be able to carry soluble substances from soil into wood. This was demonstrated for the nitrogen content of Scots pine stakes placed in sterilised, nitrogen-augmented soil (Uju *et al*, 1981)

Baines (1983) found that there was a 40% increase in the nitrogen content at the ground line of partially buried Scots pine stakes, despite this region being sealed. Furthermore, there was no evidence of microbial colonisation in this zone, indicating that nitrogen had been taken up from the soil by the lower zones of the stake and transported through the wood by wick action. However, while the rate of wick action in stakes placed in wet soil (26% moisture content) was six times that measured in moist soil (20% moisture content), the nitrogen increase of the former group of stakes was far from being six times as high. Other workers who have measured substantial

nitrogen increases in wood blocks in soil contact concluded that movement of soluble nitrogen from soil into wood was probably negligible (King *et al*, 1976; Waite and King, 1979). All wood blocks placed in soil contact in the work reported here were totally buried in soil, eliminating the possibility of wick action (Uju *et al*, 1981 *op cit*).

A number of workers have demonstrated the presence of nitrogen fixing bacteria in wood in soil contact (Sharp and Millbank, 1973; Sharp, 1974; Baines and Millbank, 1976), though the significance of these microorganisms on the wood nitrogen content has not been determined.

Waite and King (1980) showed that the bulk of nitrogen moving into decaying wood blocks is in the form of microorganisms. There was a continuing increase in the wood nitrogen content throughout the study which was attributed to a continued invasion of the wood by microorganisms. In pure culture studies using two basidiomycetes and a soft rot fungus, King and Waite (1979) demonstrated that all three fungi translocated considerable quantities of nitrogen to wood as part of the colonisation process. These workers also noted that the nitrogen content of blocks inoculated with the soft rot fungus were continuing to increase at the end of the incubation period, whereas values for the basidiomycetes had generally reached a maxima at the third or sixth week of the experiment. It was considered probable that once a basic nitrogen or biomass level was achieved by the basidiomycetes a continuous association with the substrate was no longer necessary. In contrast the soft rot fungi retained contact with the basal medium and continued to raise wood nitrogen levels.

A succession of microorganisms have been demonstrated to colonise untreated and CCA-treated wood in soil contact before decay commences (Butcher, 1968; Banerjee and Levy, 1971; Greaves, 1972; Clubbe and

Levy, 1982). King *et al* (1980b) demonstrated that the concentrations of bacteria commonly found in soil or decayed wood, significantly increased wood nitrogen concentrations. They also suggested that colonising bacteria may contribute significant quantities of growth factors in which wood may be deficient during autolysis.

1.4 Wood preservatives.

1.4.1 Introduction.

Wood preservatives are commonly classified into three groups:

1. Preservative oils derived from coal tar, petroleum or wood tar.
2. Water-borne chemicals.
3. Solvent type preservatives containing chemicals toxic to fungi and insects which are soluble only in organic solvents (Findlay, 1985b).

A number of reviews on all three preservative groups are available (Hartford, 1973; Wilkinson, 1979; Findlay, 1985b *op cit*), therefore further information will only be given on the two preservatives used in this work: copper chrome arsenate (CCA) and ammoniacal copper arsenate (ACA), both of which are classified as water-borne preservatives (Wilkinson, 1979 *op cit*).

The idea of using water as a carrier for preservatives has long been an economically appealing one (Hartford, 1973 *op cit*). In early formulations a means whereby the preservative salts could become fixed in the wood, while still retaining their preservative effectiveness was a problem (Hartford, 1973 *op cit*). In general, the fixative of choice is a compound of hexavalent chromium (Hartford, 1973 *op cit*); this is used for the CCA group of preservatives. An alternative to chromium is the retention of the preservative in solution by means of volatile acid or ammonia, which would be lost from the wood on exposure; this idea is used for the preservative ACA (Hartford, 1973 *op cit*).

1.4.2 Formulation of the wood preservatives.

The first CCA preservative was patented in India by Kamesam (1933) under the name "Ascu". Since the early 1930's CCA preservatives have increased in popularity (Wilkinson, 1979 *op cit*) and over 60 000 tonnes are used annually (Aston, 1985). The range of CCA preservatives has also increased, not only in the physical form in which they are supplied, but also in the varying proportions of the copper, chrome and arsenic compounds which make up the formulations (Aston, 1985 *op cit*). This varies from country to country (Wilkinson, 1979 *op cit*). The New Zealand Timber Preservation Association lists ten formulations which conform to their requirement of: copper, 20-30%; chromium, 25-45% and arsenic, 30-50% of the total (Wilkinson, 1979 *op cit*). The U.K. specification (BS 4072: 1974) contains only two compositions, which are presented in table 1.

Table 1 Nominal compositions of active ingredients (BS 4072:1974).

Ingredient	Type 1		Type 2	
	Nominal	Minimum	Nominal	Minimum
	%m/m	%m/m	%m/m	%m/m
Copper (expressed as $CuSO_4.5H_2O$)	32.6	30.0	35.0	31.5
Dichromate (expressed as $K_2Cr_2O_7$ or as $Na_2Cr_2O_7.2H_2O$)	41.0	38.0	45.0	40.5
Arsenic (expressed as $As_2O_5.2H_2O$)	26.4	24.0	20.0	18.0

CCA type 2 (table 1; BS 4072: 1974) was used in all experiments in the current work.

The original patent for an ACA wood preservative, issued to Gordon in 1939, was formulated from copper hydroxide, arsenic trioxide, acetic acid and ammonia. Basic copper carbonate is often used as a cheaper alternative to copper hydroxide (Hartford, 1975).

ACA is far less widely used as a wood preservative than is CCA, being utilised primarily in the U.S.A. and Canada (Wilkinson, 1979 *op*

cit). One reason for the use of ACA in Canada is that the treating solution can be heated, thus allowing treatments to be carried out in very cold weather (Wilkinson, 1979 *op cit*). CCA, in contrast, should not be heated above 50°C for prolonged periods because this produces insoluble precipitates similar to those formed during fixation (Wilkinson, 1979 *op cit*).

Refractory wood species, such as spruce, cannot be adequately treated with the acid preservative systems, such as CCA, that are presently in use (Clarke and Rak, 1974). A thermal diffusion treating process using ACA has been employed in an effort to improve the preservative penetration of refractory wood species (Ralph and Shields, 1984). Even without the application of heat, ACA is considered to provide better preservative penetration in the radial direction of refractory wood species than is CCA (Rak, 1977). ACA has also been demonstrated to provide preservative penetration of heartwood specimens which is equal to or better than that of CCA (Gjovik, 1983). ACA has been shown to give better penetration of toxic metals in wood cell walls (Hulme, 1979; Henningsson *et al*, 1980; Greaves and Nilsson, 1982). It has been suggested that ACA can be used to treat hardwood species for which CCA has failed to give adequate cell wall penetration (Henningsson *et al*, 1980 *op cit*; Greaves and Nilsson, 1982 *op cit*).

ACA treating solutions employed in this work were formulated according to Hulme and Butcher (1977c), having a molar ratio of 1.61 copper: 1 arsenic (Briscoe, 1987) dissolved in an ammonia solution.

1.4.3 Leaching of CCA and ACA from treated wood.

The extent of fixation of preservative metals within treated wood can be expressed in terms of the amounts of these metals which can be leached from the wood. In the literature fixation and leaching of preservative elements are frequently used as interchangeable terms, though this can easily lead to confusion (Dahlgren, 1972). Dahlgren (1972, *op cit*) proposed that the term "fixation" be used for the process of precipitation or other ways of rendering active elements resistant to removal from the wood, and "leachability" as a measure of how resistant the active elements are to removal by the action of a specified solvent, such as water.

CCA formulations similar to that used throughout this project, have been demonstrated to be highly resistant to leaching by water (Dunbar, 1962; Fahlstrom *et al*, 1967; Henry and Jeroski, 1967; Dahlgren, 1975a; Henshaw, 1979 and Plackett, 1984), although some small leach losses of the preservative metals have been reported. Leach losses of chromium have been reported to be negligible (Dunbar, 1962 *op cit*; Fahlstrom *et al*, 1967 *op cit*; Dahlgren, 1975a *op cit*; Evans, 1978; Henshaw, 1979 *op cit*; Briscoe, 1987), while losses of copper and arsenic are generally greater. Dahlgren (1975a *op cit*) found that the percentage losses of copper and arsenic were very similar, while Evans (1978 *op cit*) and Henshaw (1979 *op cit*) found that arsenic losses were greater than those of copper. In contrast, studies by Dunbar (1962 *op cit*) and Fahlstrom *et al* (1967 *op cit*) demonstrated that copper constituted the greatest percentage loss.

All CCA preservatives are non-volatile, and any losses in service must result solely from the leaching of salts in damp conditions (Wallace, 1968). Generally, the only way in which leach losses of

preservative elements from large scale in-service materials can be assessed is by analysing the preservative metal content of the treated wood, for example water cooling towers (Dunbar, 1962 *op cit*) and poles (Evans, 1978 *op cit*). It is sometimes possible to carry out analyses on the surrounding environment into which the metals have been lost, (e.g., De Groot *et al*, 1979; Henningsson and Carlsson, 1984). If this can be done, additional useful information can be obtained.

A number of leaching studies have demonstrated that copper is highly fixed within both ACA-treated softwoods (McCarthy and Wilson, 1957; Rak, 1976) and hardwoods (Da Costa, 1955). However, substantial arsenic losses have been measured on leaching ACA-treated specimens (Wilson *et al*, 1955; Da Costa, 1955 *op cit*; McCarthy and Wilson, 1957 *op cit*; Rak, 1976 *op cit*). Rak (1976 *op cit*) demonstrated that the copper:arsenic molar ratio of the ACA formulation effected the amount of arsenic leached from the treated wood, so that when the ratio was increased from 0.8:1 to 2.5:1, the leach losses of arsenic from ACA-treated white spruce (*Picea glauca*) decreased from 53 to 15%. Da Costa (1955 *op cit*) used an ACA formulation, having a molar ratio of 1.79 copper:1 arsenic, to treat blocks of *Eucalyptus regnans*, while Wilson *et al* (1955 *op cit*) and McCarthy and Wilson (1957 *op cit*) used an ACA formulation with a molar ratio of 1.4 copper:1 arsenic to treat blocks of *E.regnans* and *Pinus radiata* respectively. In all three studies the ACA-treated wood blocks lost approximately 55% of their initial arsenic retention after 128 days of leaching.

In contrast, De Groot *et al* (1979) reported only small accumulations of arsenic in soil adjacent to pine stakes treated with an ACA solution, with a copper:arsenic molar ratio of about 1.4:1.

The level of arsenic in soil immediately surrounding the ACA-treated stakes was also very similar to that determined for stakes treated with a CCA type 2 solution (De Groot *et al*, 1979 *op cit*).

Small leach losses of copper are anticipated from ACA-treated wood blocks of all three wood species in the current work, while losses of arsenic are expected to be large. The work of De Groot *et al* (1979 *op cit*) indicates that these leach study results may not be mirrored by similar large losses of arsenic to adjacent soil.

De Groot *et al* (1979 *op cit*) measured the copper, chromium and arsenic content of soil collected from around CCA and ACA treated southern pine stakes, which had been exposed in a sandy, acidic soil for 30 years; small amounts of the preservative metals were found to have accumulated in the soil proximal to the stakes. Henningsson and Carlsson (1984) found a limited leaching of arsenic and a very small loss of copper from CCA-treated timber in children's playground equipment. A small proportion of the leached chemicals had accumulated in sand close to the timber, but were considered not to represent a hazard to children (Henningsson and Carlsson, 1984 *op cit*).

The soil copper content ranges from 1-3ppm in copper deficient soils, to 200ppm or more in soils where excess copper has accumulated (Fiskell, 1965). However, the availability of soil copper to plants is relatively low (Fiskell, 1965 *op cit*). Yamamoto *et al* (1985) added various concentrations of copper sulphate solution to soil (between zero and 1600ug copper g^{-1} dry soil), and found that only small quantities of this added copper were removed by water extraction (between 0.3 and 200ug copper g^{-1} dry soil), though extraction with 1 molar hydrochloric acid gave much higher values (12-1850ug copper g^{-1} dry soil). Levi *et al* (1974) found that grape vines adjacent to CCA-treated posts had no measurable increase in amounts of copper,

chromium or arsenic in fruit, leaf or stem tissue, even when the posts had been inserted in the ground before they had dried.

There is no evidence that the small amounts of preservative metals which have accumulated in soil adjacent to CCA and ACA-treated material represent any hazard to animal or plant life. Furthermore increased preservative metal levels in the soil appear to have no sterilising effect on the microbial population, though a selection effect, leading to an increase in the numbers of copper tolerant fungi has been observed (Murphy, 1982; Yamamoto *et al*, 1985 *op cit*).

1.4.4 Fixation of CCA and ACA within treated wood.

The fixation mechanism of CCA to wood has been presented in a series of papers by Dahlgren and Hartford (1972a, b and c) and Dahlgren (1972, 1974, 1975a, b) and by Pizzi (1981, 1982a, b and c) and the mechanisms presented by both groups of workers have been critically reviewed by Plackett (1983).

Dahlgren and Hartford studied the changes in pH when known volumes of CCA solution and sapwood sawdust were mixed and stored at constant temperature (20°C). The chromium fixation rate was determined by X-ray analysis of leached sawdust samples at set time intervals following the initial treatment. Data from similar work by Wilson (1971) were used to evaluate the influence of preservative type on chromium fixation (Dahlgren and Hartford, 1972c *op cit*). The pH of CCA treated sawdust was seen to increase gradually to a maximum and then to oscillate over a period of several months (Dahlgren and Hartford, 1972a *op cit*). From this information and the X-ray analysis results Dahlgren and Hartford suggested the following course of fixation (summarised by Dahlgren, 1972 *op cit*) -

CCA preservatives in contact with wood result in an instant

extensive increase of pH, due to ion-exchange and adsorption reactions with the wood (the period of momentary initial reactions). During precipitation of the active elements the pH continuously increases before reaching a maximum upon total chrome consumption (the period of primary precipitation fixation). Some of the early reaction products are unstable and slowly dissolve forming stable compounds. The conversion proceeds by proton liberation and proton consumption reactions. Depending on which of these reactions predominate, the pH either increases or decreases, producing the pH oscillations (the period of conversion reactions). The final equilibrium fixation products are: ion-exchange fixation of copper to wood, $CrAsO_4$, $Cu(OH)CuAsO_4$ and $Cr(OH)_3$.

Dahlgren and Hartford (1972a *op cit*) stated that, "The presence of wood drastically changes the precipitation conditions, as is shown in this investigation, so precipitation studies from solutions only cannot possibly give a picture of what actually happens.". Plackett (1983 *op cit*) suggests that this sentence summarised the philosophy of Dahlgren and Hartford's work, and commented that these workers were convinced of the necessity to work with wood when attempting to investigate the mechanism of CCA fixation. In contrast, Pizzi studied the interaction of CCA or CCA component elements with cellulose and lignin model compounds or with milled wood flour.

Plackett (1983 *op cit*) summarised the main features of the CCA reaction mechanisms developed by Pizzi as follows. The initial instant reactions of CCA with wood are considered to be ion exchange of copper accompanied by temporary adsorption of CrO_3, which are followed by the main fixation period involving the following reactions:

- CrVI adsorption on cellulose.
- CrVI reduction to CrIII on cellulose sites.

- $CuCrO_4$ formation and complexation with lignin guaiacyl units.
- CrVI reduction to CrIII and complexation as $CrAsO_4$ with lignin guaiacyl units or precipitation onto cellulose.
- $Cr_2O_7^{2-}$ and $HCrO_4^-$ complexation with lignin followed by CrO_4^{2-} complexation with lignin.
- Cu^{2+} complexation with lignin and cellulose.

Pizzi argued that copper arsenates are only involved as a final fixation product when a preservative of high arsenic content is used.

Pizzi (1983) suggested that the wood treating temperature affected both the rate of fixation reactions within the wood and the distribution of chrome (and of copper and arsenic chemically reacted with it) between lignin and holocellulose (cellulose and hemicelluloses, Scheffer, 1973). Thus at wood treating temperatures of 20 and 80°C the approximate distribution of chrome between holocellulose and lignin will be approximately 35:65% and 50:50% respectively. Since preservative treatments with CCA are normally carried out at ambient temperatures, this suggests that in most CCA-treated material the majority of the preservative metals will be fixed to the lignin, rather than to the holocellulose.

Both Dahlgren and Hartford and Pizzi have, by necessity, employed models dissimilar to the situation found in treated wood. Plackett (1983) commented that fixation "sites" for CCA preservative elements in wood may differ depending upon whether sawdust is simply mixed with CCA solution (as in Dahlgren and Hartford's work) or whether wood is vacuum-impregnated and subsequently ground for experimental purposes. This must also be true for CCA mixed with model compounds or wood flour. It is notable that Pizzi carried out his studies at a range of temperatures (generally 26 to 80°C) and many of the calculations he presented were derived from the 80°C results. This

casts doubt on the validity of Pizzi's model, as CCA treatments are typically carried out at ambient temperatures and it is recommended that CCA should not be heated above 50°C (Wilkinson, 1979).

The fixation mechanism of ACA differs markedly from that of CCA (Hulme and Butcher, 1977c). It is thought to be accomplished by the precipitation of water-insoluble salts as the ammonia evaporates (Hulme, 1979). Kuperman *et al* (1955, cited by Hulme, 1979 *op cit*) found that, in the absence of wood, a wide range of complexes (varying in the ratio of copper oxide to arsenic oxide) were formed by mixing copper salts with arsenates or arsenites in aqueous ammonia. However, in the presence of wood fixation is more complex (Hulme, 1979 *op cit*). For example, copper complexes can be formed with cellulose (Vazirani and Narwani, 1969).

It had been assumed that during the fixation of ammonia-based wood preservatives, such as ACA, ammonia was lost from the wood (Ruddick, 1979). However, Ruddick (1979, *op cit*) demonstrated that the nitrogen content of ACA treated wood was substantially increased after treatment with the preservative and Sundman (1984) suggested that in ACA systems copper is probably fixed to wood as $Cu(NH_3)n(OH)_2$, where n=0-3. Ruddick (1979 *op cit*) observed that the ammonia penetration into white spruce (*Picea glauca* [Moench] Voss) poles was greater than that of copper and arsenic. This indicates that only a percentage of the ammonia remains associated with the copper in the wood. Increasing the nitrogen content of wood blocks has been shown to increase the rate of decay in pure culture tests (Savory, 1954a, 1954b; Levi and Cowling, 1969) and in soil burial studies (Waite and King, 1979). Toxic values of CCA also increase when the nutrient status of the wood substrate is increased (King *et al*, 1981b). Sundman (1984) and Briscoe (1987) found that treatment of wood with ACA increased the toxic values of copper when

compared with values for CCA-treated wood. However, Hulme and Butcher (1977c) found no difference in toxic values of copper for CCA and ACA-treated wood.

The reactions between cuprammonium ions and wood constituents effectively remove cations from later reactions such as precipitation with arsenate upon evaporation of ammonia (Hulme, 1979). The moderate resistance to leaching of arsenic in ACA-treated wood blocks lends support to the theory that in a preservative of this type copper reacts preferentially to form complexes which do not involve arsenic (McCarthy and Wilson, 1957).

1.4.5 Selective absorption.

BS 6009 (1982) recommends that for water-soluble preservatives the retention of preservative metals should be calculated from the mass of preservative absorbed by each specimen, and the concentration of the solution. However, a number of workers (Smith and Williams, 1973 ; Henshaw, 1979; King *et al*, 1981b) have noted that wood treated with CCA often contains higher concentrations of preservative elements than had been predicted by the liquid uptake method. The difference between the two methods of preservative metal content determination varied for the three metals, therefore, the effect has been referred to as selective absorption (Smith and Williams, 1973 *op cit*). It has been suggested (Smith and Williams, 1973 *op cit*; Henshaw, 1979 *op cit*) that selective absorption may be attributable to adsorption, precipitation, chemical reactions between the preservative and specific wood components or a combination of the three.

Other studies (Fahlstrom *et al*, 1967; Henningsson *et al*, 1980) reported good correlations between analytical data and calculated

retentions, indicating that no selective absorption had occurred. ACA, as well as CCA, treated material was included in the latter study.

1.4.6 Mode of action of CCA and ACA.

The mechanism of CCA preservative action in the wood cell wall is unclear (Hale and Eaton, 1986). For a multi-salt preservative like CCA it is probable that its toxicity lies in the concerted action of all three toxic elements, copper, chromium and arsenic, as even the most resistant fungi are only tolerant to one of the three metals (Chou *et al*, 1973).

Laboratory and field studies indicate that the main toxicant in CCA limiting the growth of soft rot fungi is copper (Hulme and Butcher, 1977a). Although less data is available on the fungal toxicants of ACA, it is likely that copper is also the main fungal toxicant in this case. Hulme and Butcher (1977c) employed ACA on this basis in their work.

The selection of copper tolerant fungi observed in soil adjacent to CCA-treated wood, and within the treated wood (Murphy, 1982) suggests that the preservative is exerting a fungistatic or fungicidal effect on the copper intolerant portion of the microbial population. Although copper is essential for fungi at trace concentrations, higher levels have long been noted for their high toxicity and value as fungicides (Ross, 1975). The available evidence suggests that copper is rapidly adsorbed to the fungal cell surface and to the conidia, and is subsequently transported into the cells (Ross, 1975 *op cit*). Although initially the effect of copper on the conidia is fungistatic, it becomes fungicidal after a few hours

(Ross, 1975 *op cit*). The nature of the fungitoxicity of copper is unclear, though there is some evidence that copper interacts with the genetic apparatus of the fungi (Ross, 1975 *op cit*).

Despite the presence of copper in CCA-treated wood, soft rot fungi have been observed colonising CCA-treated softwoods and hardwoods (Clubbe and Levy, 1982), and in the latter case soft rot decay occurs at relatively high preservative retentions (Hulme and Butcher, 1977c). Nilsson (1982) suggested that, of the four stages of soft rot attack (see section 1.3.1), CCA did not prevent colonisation of the wood substrate, hyphal penetration of the wood fibre walls, or the formation of soft rot cavities around oriented hyphae. He proposed that CCA treatment causes changes in the wood structure that masks the substrate (the carbohydrate), so that it cannot be recognised by the penetrating hyphae. Thus, T-branching is prevented and no soft rot cavities will be formed (Nilsson, 1982 *op cit*).

Soft rot decay fungi capable of degrading CCA-treated wood are generally recognised as possessing some metal tolerance (Daniel and Nilsson, 1988). Levi (1969) found that fungal secretions of a number of white and brown rot fungi resulted in the dissolution of copper, chromium and arsenic in CCA-treated wood. Two of the copper tolerant fungi employed by Levi (1969 *op cit*) precipitated a proportion of the soluble copper as relatively non-toxic copper oxalate. Murphy (1982) found that certain copper tolerant mould fungi could detoxify copper in agar, and this was also by the conversion of copper to copper oxalate.

Daniel and Nilsson (1988 *op cit*) investigated the decay of CCA-treated birch (*Betula verrucosa*) by *Phialophora mutabalis*, a soft rot decay fungus with a known capacity to degrade CCA-treated wood. They found that the fungus was capable of degrading the entire S2 layer and occasionally the S1 layer of the fibres and ray cells,

while the tertiary wall/CCA layer of precipitates remained apparently undegraded. The diffusion of the agents of decay (enzymes/radicals) was not affected by the local CCA wood cell wall concentration, nor was there any indication of their inhibition by CCA.

Daniel and Nilsson (1988 *op cit*) found a concentration of preservatives in residues (granullar and fibrillar) during the degradation process. The fibrillar residues were thought to represent products of cell wall degradation and may have contained phenolic materials (Daniel and Nilsson, 1988 *op cit*). The association of these residues with CCA is consistent with the preferential binding of CCA preservative elements to lignin in wood (Pizzi, 1982c). Daniel and Nilsson (1988 *op cit*) suggested that the *P.mutabalis* may have a capacity for selectively removing the wood carbohydrate, leaving lignin (presumably modified) and at least some of the bound CCA. The granular residues were thought to consist of melanin-type material of fungal origin and CCA. Melanin type compounds have considerable activity as cation exchange materials (White, 1958, cited in Daniel and Nilsson, 1988 *op cit*) and were considered to have bound the preservative metals (Daniel and Nilsson, 1988 *op cit*). Copper and possibly arsenic were detected intracellularly within the hyphae, particularly in the storage bodies. However, far greater levels were externally distributed in the residues around the hyphae. This suggests that the extracellular binding of CCA to fungal derived, or products of wood cell wall breakdown is more important than intracellular metal binding in the soft rot attack of CCA-treated wood (Daniel and Nilsson, 1988 *op cit*).

1.4.7 Efficacy of CCA and ACA.

CCA and ACA have been reported to be effective wood preservatives both in marine (Ruddick, 1987) and soil environments (Davidson, 1977; Gjovik and Gutzmer, 1983). However, a number of workers have commented on the relatively poor performance of some CCA treated hardwoods in ground contact, when compared with the softwoods (Dickinson, 1974; Hulme and Butcher, 1977a; Butcher, 1978). Reports from countries such as Australia, Ghana, Nigeria, Papua New Guinea, South Africa, and South East Asian countries indicate that soft rot decay in the treated zone of hardwood poles is causing a reduction in service life (Greaves and Nilsson, 1982).

Studies of the macro and micro-distribution of the preservative elements in CCA-treated hardwoods and softwoods have shown substantial differences in the preservative distribution between the two wood types (Greaves, 1972, 1974; Dickinson, 1974 *op cit*; Dickinson *et al*, 1976). In softwoods the various preservative components were found to be very evenly distributed across the various tissues, and all wall layers of the tracheids were treated in depth, with relatively high levels of preservative (Greaves, 1974 *op cit*; Dickinson *et al*, 1976 *op cit*). The distribution of preservative metals in a number of CCA-treated hardwoods was reported to be very uneven (Greaves, 1972 *op cit*, 1974 *op cit*; Dickinson, 1974 *op cit*; Dickinson *et al*, 1976 *op cit*). Large accumulations of the preservative metals were measured in the rays and vessels of the treated hardwoods, but the fibres had received far lower treatment, with little preservative being measured in the fibre walls, particularly the S2 layer (Greaves, 1972 *op cit*, 1974 *op cit*; Dickinson, 1974 *op cit*; Dickinson *et al*, 1976 *op cit*; Greaves and

Nilsson, 1982 *op cit*). The soft rot fungi are thus able to grow in the relatively unprotected wood cells, some distance from the main concentrations of the preservative (Greaves and Nilsson, 1982 *op cit*).

The differences in macro and micro-distribution of CCA in softwoods and hardwoods have been used to explain the differences in their soft rot decay susceptibility. This explanation is known as the microdistribution theory (Greaves, 1972 *op cit*, 1974 *op cit*; Dickinson, 1974 *op cit*) and is accepted as being one explanation of the phenomenon (Greaves and Nilsson, 1982 *op cit*). However, Hulme and Butcher (1977a) found only a two-fold difference in the copper content of the S2 layers of the softwood (*Pinus radiata*) and hardwood (*Betula alba*) examined, though an 8-fold difference in susceptibility to soft rot existed between the two species. Therefore differences in microdistribution of preservative metals cannot account for all of the difference in soft rot susceptibility of CCA-treated hardwoods and softwoods.

Greaves and Nilsson (1982 *op cit*) examined the degree of soft rot attack of various field test hardwood specimens treated with a number of CCA formulations and with ammoniacal copper-chrome-arsenic. They found that copper distribution throughout the wood was more homogeneous in the ammoniacal CCA treatment, and soft rot more limited. The reduced level of soft rot was ascribed to the more effective distribution of the toxicant in the ammoniacal CCA-treated wood. This was thought to be due to the wall swelling action of ammonia allowing a more effective penetration of preservative metals into the cell walls (Greaves and Nilsson, 1982 *op cit*). A similar improvement in microdistribution of preservative metals in hardwood fibres and also in subsequent resistance to degradation by soft rot fungi was observed by Henningsson *et al* (1980) for hardwood stakes

treated with various copper containing ammoniacal formulations. Henningsson *et al* (1980 *op cit*) found that these improvements only occurred in material for which drying had been delayed for a three week period.

Hardwoods are more susceptible to attack by soft rot fungi than are softwoods (Hulme and Butcher, 1977c). This was attributed to the wood substance of the hardwoods being a more available substrate for these fungi, and consequently more toxicant may be needed to prevent soft rot attack in the hardwoods. Butcher and Nilsson (1982) extended this explanation to include the variable susceptibility of wood species to soft rot and the variable performance of CCA in controlling decay. They suggested that wood species with lower lignin contents (hardwoods in general) are more prone to soft rot attack than species with higher lignin contents (softwoods in general). Presuming that lignin provides the major fixation sites for copper in CCA-treated wood, as suggested by Pizzi (1982c), wood of low lignin content will have a copper retention level which is less than the toxic threshold for soft rot fungi (Butcher and Nilsson, 1982 *op cit*). However, in wood of high lignin content, the fixation of copper is enhanced and retention levels in the S2 layers are well in excess of toxic thresholds (Butcher and Nilsson, 1982 *op cit*).

Nilsson (1982) observed that if T-branching of the soft rot fungi was prevented no soft rot cavities were formed. He suggested that the fungal hyphae are induced to form T-branches in the S2 layer by the carbohydrate fraction of the wood substrate, but induction is unlikely to be caused by lignin. Rather, lignin could act as a masking compound of the T-branching initiation (TI) sites, preventing the fungi from recognising its substrate (Nilsson, 1982 *op cit*). Nilsson (1982 *op cit*) further proposed that CCA treatment could cause changes in the wood structure and consequently further mask the TI

sites.

Butcher and Nilsson (1982 *op cit*) suggested that, not only was the amount of lignin present in the wood important in determining its soft rot susceptibility, but also that wood species containing guaiacyl lignin are more resistant to soft rot than those containing syringyl-guaiacyl lignin. Nilsson *et al* (1988) considered that only timber species with a high lignin content and a low syringyl:guaiacyl ratio should be selected for long term exposure in ground contact if long lasting performance is required. While softwoods would tend to meet this requirement, only a few species of hardwoods, such as Tasmanian myrtle (*Alstonia scholaris*) and maple (*Acer negundo*) have the very high lignin contents and low syringyl:guaiacyl ratios suggested (Nilsson et al 1988 *op cit*).

Butcher (1980) reported soft rot in a number of CCA treated *Pinus radiata* specimens. This was unexpected, since the copper retentions of the specimens were higher than reported toxic values for pine and the material had not been in service long (3 to 10 years). While the decayed posts and poles were in several different locations in New Zealand, all the material had been embedded in concrete. One of the by-products formed during the setting reactions of concrete is free calcium hydroxide, which leaches from concrete over a relatively long time period (Butcher, 1980 *op cit*). Other calcium compounds are also present in concrete mixtures or hardened concrete (Murphy, 1983).

Butcher (1980 *op cit*) carried out a leaching trial on CCA treated pine using both deionised water and a saturated calcium hydroxide solution. He found losses of all three preservative metals in the latter case, though only arsenic leach losses were apparent when deionised water was employed. While some loss of copper was noted in the decayed CCA-treated material, retentions of copper were always greater than the toxic threshold values for pine (Butcher, 1980 *op*

cit) therefore, losses of preservative metals alone cannot account for the unexpected soft rot decay.

Murphy (1983) found that calcium compounds reduced the toxicity of CCA to a soft rot fungus (*Humicola* sp.) at copper to calcium ratios of 1:1 and 1:10 using a cellulose filter paper technique. This result may indicate that calcium compounds can reverse the toxicity of copper to microorganisms (Murphy, 1983 *op cit*). The Building Research Association of New Zealand recommended in 1982 that a bituminous coating or plastic wrap be given to parts of building poles that come into contact with concrete to protect them from any influence that the concrete might have on the durability of the treated wood (cited in Murphy, 1983 *op cit*).

The premature decay of CCA-treated horticultural pine posts was first identified in New Zealand in 1982 (Butcher, 1984). Many types of decay were shown to be associated with this premature decay, with most failures being due to brown rot fungi, though the major decay type present in the posts was soft rot (Butcher, 1984 *op cit*). Higher concentrations of ions, such as calcium, potassium and magnesium, than are normally found in pastoral soils were present in the horticultural soils (Plackett, 1984). Inorganic salt solutions, containing calcium, potassium and magnesium ions, caused enhanced copper leaching relative to leaching with deionised water (Plackett, 1984 *op cit*). Losses of copper were greater from the relatively low chrome formulations (used in New Zealand since the late 1960's), than were measured from the higher chrome formulations, similar to that employed in the current work (Plackett, 1984 *op cit*). Although some loss of preservative metals was measured from the below ground portion of the failed posts, the minimum preservative loading for this commodity was still generally met (Butcher, 1984 *op cit*), thus leach losses of preservative metals could not be solely responsible

for the early failure of the posts. An accumulation of calcium salts measured in the below ground zones of some treated posts (Butcher, 1984 *op cit*), may account for the reduced toxicity of the copper remaining in these posts (Murphy, 1983).

Horticultural soils represent a particularly high hazard for CCA treated material due to their high nutrient content and broad spectrum of decay organisms present, as well as their high ionic status (Butcher, 1984 *op cit*). The premature decay of the CCA-treated pine posts is likely to be due to a combination of all these factors, as well as to the preservative formulation used. Altering the formulation of CCA employed to a relatively high chrome formulation and/or increasing the preservative retention employed have been suggested as methods of improving the performance of CCA treated softwoods in horticultural situations (Butcher, 1984 *op cit*).

Pizzi and Conradie (1986) and Pizzi *et al* (1986) have recently focussed attention on the role of tannins in the susceptibility of CCA treated wood to soft rot decay. The tannin content of timber varies according to the species, though hardwoods generally have a much higher tannin content than softwoods (Pizzi *et al*, 1986 *op cit*). In the hardwoods flavonoid tannins are concentrated in the rays, on the surface of the S3 membrane and within the primary and secondary cell wall layers, particularly the S2, tightly complexed to the holocellulose in them.

The polyflavonoid tannins complex heavy metal ions and are considered to be more reactive than lignin and wood carbohydrates (Pizzi *et al*, 1986 *op cit*). Dahlgren (1975b) observed that eucalyptus (a high tannin species) had a very short primary fixation period at lower preservative concentrations (2 and 2.5% CCA). He suggested that some highly reactive reducing matter could be responsible for this, although the availability of this reactive matter seemed to be

limited, since the primary period was fairly long at the higher concentration levels (4 and 5% CCA). Pizzi *et al* (1986 *op cit*) proposed that where the tannin deposits are present on the S3 surface in the lumen and in the rays, CCA will complex with the tannins before the bulk of it can penetrate the fibre walls. The tannins having structural functions in the cell walls will also "pinch" some of the remaining CCA which penetrates the cell walls, leading to the microdistribution of preservative elements observed in the hardwoods (Greaves, 1972, 1974, Greaves and Nilsson, 1982). However, uneven microdistribution of preservative elements has been associated with a number of hardwood species which did not exhibit a rapid primary fixation period when tested by Dahlgren (1975b*op cit*), e.g. birch (*Betula* sp.) (Dickinson *et al*, 1976).

Pizzi *et al* (1986 *op cit*) were of the opinion that even small amounts of tannin cause severe undertreatment of the structural wood constituents which in turn adversely affects the long term durability of CCA treated timber. They proposed that the total mechanism of resistance and failure is due to the balance of distribution of reactions among the various proportions of highly reactive tannins and the more abundant, but less reactive, lignin and carbohydrates present in any wood. Among softwoods radiata pine from New Zealand has a tannin content higher than other common pine species (Pizzi *et al*, 1986 *op cit*). Pizzi *et al* (1986 *op cit*) associated this with the premature failure of CCA-treated radiata pine stakes in horticultural soils (Butcher, 1984). However, when Scherer and Baecker (1988) investigated the effect of tannin on CCA efficacy using *Pinus patula* (with and without 4%w/w tannins added) and *Eucalyptus grandis* (with and without natural tannins) they concluded that the tannins had no effect on CCA efficacy at the preservative retentions tested.

1.5 Techniques used in the investigation of CCA and ACA wood preservatives.

A wide range of techniques can be used in the investigation of various aspects of the performance of untreated and preservative treated woods. The techniques which are commonly used (often in conjunction with one another) or have proved most useful are discussed in this section with attention focused on those employed in this study.

1.5.1 Testing of wood against microorganisms.

The soft rot fungi have been shown to cause the majority of decay at the wood/soil interface (see section 1.3.1). Therefore, in investigating microbial and chemical changes in this region, it was desirable that optimal conditions for soft rot decay be used.

Soft rot fungi and Basidiomycetes are physiologically and biologically different (Savory, 1954 a and b; Savory and Pinion, 1958). Therefore, it is not surprising that laboratory test methods developed for testing preservative treated wood samples against Basidiomycete attack, such as the soil block method (Behr, 1973; Amburgey, 1978), were unsuitable for assessing the natural durability of wood species and the efficacy of various potential preservatives against soft rot attack (Bravery, 1968b). Agar tests and soil burial tests, which employ either inoculated sterile soil or vermiculite, or alternatively unsterile soil have been developed for assessing sample performance against soft rot. More recently fungal cellar or soil bed tests have been used to assess preservative performance against soft rot attack (Hedley, 1980; Baines, 1982; Hill *et al*, 1986).

Unsterile soil burial methods to evaluate anti-soft rot

efficiency of wood preservatives are said to be poorly reproducible (Gersonde and Kerner-Gang, 1976). Gersonde and Kerner-Gang (1976 *op cit*) proposed using a vermiculite burial method with a solution containing fungal spores as the inoculum, while Hilditch (1978) recommended using a test "portfolio" of soil types. The latter test would allow soil variations to be accounted for and may identify soil types in which a preservative fails, and those in which it performs well.

In natural conditions, soft rot decay of timber in the ground is the result of the action of many different organisms (Bravery, 1975) and no one species is solely responsible (Wilkinson, 1979). Unsterile soil burial tests are generally favoured for soft rot testing because any preservative tested is subjected to the influence of a natural soil microflora, thus greater attack of woods occurs (Savory and Bravery, 1970). In unsterile soil different groups of organisms are likely to be active, such as bacteria, cellulolytic microfungi and Basidiomycetes. In soil burial studies Savory and Bravery (1970, *op cit*) found that, while decay due to soft rot fungi had occurred, no evidence of Basidiomycetes was found.

Testing of wood preservatives using natural soils involves the use of soil as the source of moisture, nitrogen and other essential nutrients and also as the inoculum source (King *et al*, 1981a). Unsterile soil burial experiments also have the advantage that longer incubation periods can be used, since remoistening the soil does not carry the risk of contamination (Savory and Bravery, 1971). The unsterile soil burial technique was employed in this project.

Durability of untreated wood, and/or chemical effectiveness of a preservative is normally assessed by estimating fungal attack, measured as weight loss of test blocks after the required incubation period (Bravery, 1975). The effectiveness of a preservative is

expressed using its toxic values, which define the concentration range of the preservative which just inhibits decay and that which just permits it (Bravery, 1975 *op cit*).

Using weight loss as a criterion for microbial decay may make it necessary for lengthy incubation periods (Savory and Bravery, 1971), thus the measured loss of strength has been used in an attempt to develop a more sensitive test (Smith, 1970; 1980; Baines, 1982; Ruddick, 1986). However test block selection is more critical when a strength loss criteria is employed (Savory and Bravery, 1970 *op cit*) as the moisture content of wood can affect its strength (Baines, 1982 *op cit*), therefore the weight loss criterion is considered more convenient (Bravery, 1975). As a small amount of weight loss is always caused by factors other than fungal attack (Bravery, 1975), a 3% weight loss is generally used as the minimum criterion of decay (Bravery, 1968; Savory and Bravery, 1970; Bravery, 1975; BS 6009, 1982). Hulme and Butcher (1977c) observed no soft rot cavities in test blocks which had less than a 3% weight loss, so 3% minimum weight loss requirement for decay by soft rot fungi seems reasonable. In this project weight loss was used as a measure of fungal attack.

The moisture content of the wood must be greater than the fibre saturation point before most fungal decay will take place (section 1.3.2). Therefore the moisture contents of wood blocks in soil burial studies are generally monitored (e.g., Mowe, 1983; Gray, 1986; Briscoe, 1987; Nayagam, 1987). Moisture contents are reported in this study.

Preservative leaching is inevitable in situations where most soft rot occurs, i.e. in soil or free water environments (Savory and Bravery, 1971). In soft rot tests of this type pre-conditioning by leaching of all preservative treated wood samples prior to their incubation has been recommended by some workers (Savory and Bravery,

1971 *op cit*; Baines, 1982; Hedley and Butcher, 1985), though it is not always carried out (Murphy, 1982; Briscoe, 1987; Nayagam, 1987). Leached wood blocks were used in some of the experiments carried out during the current work.

1.5.2 Monitoring microbial populations in wood and soil.

The ideal technique for monitoring changes in microbial populations of wood in soil contact and of the adjacent soil should fulfil the following requirements:

1. be applicable to both wood and soil samples so that results from each group could be compared.

2. detect and measure changes in the general microbial population, rather than in sections of it.

3. allow the handling of a large number of samples in a relatively short time.

4. be capable of utilising only a small portion of the wood blocks used, so that the remainder could be employed in other analyses, e.g. weight loss, moisture, nitrogen and preservative metal content determinations.

There are three main methods of assessing the microbial population of soil: cultural studies, direct examination and activity measurements (Campbell and Berkeley, 1979). These methods have also been used to assess microbial populations in wood (Murphy, 1983; Clubbe and Levy, 1982; Mowe, 1983).

Previous studies carried out on wood and adjacent soil samples (Murphy, 1982; Clubbe, 1983) have employed selective media to isolate fungi, however these techniques are prone to error, particularly when used to assess microbial populations in the soil. This is due to a combination of factors, including the presence of spores, and the

selectivity of the media employed (Campbell and Berkeley, 1979 *op cit*). Isolation techniques are invaluable in certain studies, for instance to demonstrate an increase in copper tolerant fungi around CCA-treated material (Murphy, 1982), or to relate to decay features observed within the wood (Clubbe, 1983). However, since the media used is selective, they cannot monitor changes in the entire microbial population and therefore isolation techniques were not considered suitable for use in the current work.

Microscopic examination has been used to measure changes in fungal hyphal length and bacterial numbers in soil adjacent to wood blocks (Mowe, 1983), and the presence of microorganisms in wood blocks (Clubbe, 1983; Clubbe and Levy, 1982). However, errors with this technique arise from the inability of most staining methods to discriminate between organisms that are viable when the sample was taken and those that are dead (Jackson, 1975). Jackson (1965, cited in Jackson, 1975 *op cit*) found that dead hyphae in contact with an unsterile soil were recovered intact and apparently little changed after 3-6 months. This indicates a degree of persistence of fungal cell walls that makes it certain that many hyphae seen in such a soil must be dead (Jackson, 1975 *op cit*). Therefore, microscopical observation was not considered sufficiently sensitive to detect rapid changes, particularly decreases, in microbial biomass.

Changes in microbial populations can be monitored by microbial metabolic activity measurements. In this work metabolism refers to the whole array of degradative and biosynthetic reactions taking place within cells (Brock, 1979). Soil metabolic activities are mediated by specific enzyme systems, the majority of which are produced by the soil microbiota (Barkay *et al*, 1986). Thus, measuring enzymic activity is a means of examining metabolic activity. Consequently, levels of dehydrogenases, amylases, phosphatases and

cellulases are all indicative of the major turnover processes in soils (Barkay *et al*, 1986 *op cit*). Enzymatic activity measurements are included in some toxicity assessment programmes, the most commonly used being dehydrogenase and phosphatase activities (Barkay *et al*, 1986 *op cit*). In addition to monitoring levels of specific enzyme systems other aspects of metabolic activity can be assessed, these include the measurement of levels of respiration and of concentrations of various key metabolites, such as ATP (Campbell and Berkeley, 1979 *op cit*).

Respirometry techniques have been used in many soil studies (Howard, 1972; Jenkinson and Powlson, 1976; Sparling *et al*, 1981), and have been proposed for use in wood preservative toxicity evaluation (Smith, 1969b; Behr, 1972). The respirometry studies on the decaying wood blocks used small samples (Smith, 1969b *op cit* and Behr, 1972 *op cit* used wood blocks measuring approximately 20*20*20mm), though they were not as small as the samples proposed for use in this work. Furthermore, the previous work on wood respirometry employed wood decayed by pure cultures of Basidiomycete fungi. Therefore it was not known whether this technique would be successful in measuring microbial metabolic activity in small samples of wood exposed to soft rot fungi.

Changes in microbial activity in wood blocks in ground contact, and the adjacent soil have been monitored using a dehydrogenase assay (Mowe, 1983). Ross (1970) stated that soil dehydrogenase activity appears to be more dependent upon the metabolic state of the soil than upon the activity of specific free enzymes acting on a particular substrate. Dehydrogenase activities are therefore likely to be affected by many environmental properties (Ross, 1970 *op cit*). Dehydrogenases are believed to be intracellular, non-adaptive enzymes, therefore it is not necessary to add a substrate to measure

their activity, thus avoiding preferential stimulation of any group of organisms (Howard, 1972; Bolton *et al*, 1985). The dehydrogenases are unspecific enzymes produced by soil organisms which catalyse the transfer of hydrogen from organic substances to molecular oxygen (Ruhling and Tyler, 1973).

To measure dehydrogenase activity the soil sample is mixed with an artificial hydrogen/electron acceptor, usually 2,3,5 triphenyl-tetrazolium chloride (TTC), under anaerobic conditions (Barkay, *et al*, 1986). The method assumes that, in the absence of oxygen, TTC acts quantitatively as the terminal hydrogen/electron acceptor for dehydrogenase systems, with the formation of red triphenyl-tetrazoliumformazan (TPF):

$TTC + 2H^+ + 2e^- = TPF + HCl$ (Benefield *et al*, 1977).

The TPF formed is extracted from the soil using an organic solvent, such as methanol, and its concentration is then measured colourimetrically (Barkay, *et al*, 1986 *op cit*).

The TTC assay has been used to measure dehydrogenase activity in several soil studies (Casida *et al*, 1964; Ross, 1970, 1971; Ruhling and Tyler, 1973; Sparling *et al*, 1981; Mowe, 1983; Baruah and Mishra, 1984; Bolton *et al*, 1985; Reddy *et al*, 1987). Increased levels of soil dehydrogenase activity have been associated with greater nutrient levels (Sparling *et al*, 1981 *op cit*; Baruah and Mishra, 1984 *op cit*) and with increased levels of other enzymes, such as urease and phosphatase (Bolton *et al*, 1985 *op cit*). Greater levels of dehydrogenase activity have also been measured in the rhizosphere (Reddy *et al*, 1987 *op cit*) and in soil adjacent to decaying wood blocks (Mowe, 1983 *op cit*). Reductions in dehydrogenase activity have been observed in heavy metal polluted soils (Ruhling and Tyler, 1973 *op cit*; Reddy *et al*, 1987 *op cit*). Mowe (1983 *op cit*) also demonstrated enzymic activity in small samples of decaying, 0.5%w/v

CCA-treated lime blocks, though no activity was measured in samples of non-decaying, 0.5%w/v CCA-treated lime blocks.

Since TTC is acting as an alternative hydrogen/electron acceptor to oxygen a close relationship between dehydrogenase activity and oxygen uptake of soils might be expected (Howard, 1972). However, Howard (1972, *op cit*) and Benefield, *et al* (1977 *op cit*) found considerable discrepancies between the two, with far lower levels of TPF being measured than those predicted from the oxygen uptake values obtained. Benefield *et al* (1977 *op cit*) stated that TTC may not be a very efficient hydrogen/electron acceptor and in tests they also employed a second tetrazolium salt: 2-p-iodophenyl-3-p-nitro-phenyl-tetrazolium chloride (INT), as the hydrogen/electron acceptor, since it had been shown that this compound competed well with oxygen for liberated electrons. Although dehydrogenase measurements using the INT acceptor were better than those obtained with TTC, 90% of the oxygen uptake determined was still unaccounted for (Benefield *et al*, 1977 *op cit*). Despite the possible inefficiency of TTC as an artificial electron acceptor, the assay has demonstrated changes in soil microbial activity which can be related to increases in nutrients and pollutants in the soil. Furthermore estimates of dehydrogenase activity using the TTC method are simple to carry out (Ross, 1971) and do not require any specialised equipment; only an incubator, a centrifuge and a spectrophotometer are required. Thus the TTC dehydrogenase assay was chosen to monitor changes in the microbial population of wood in soil contact and of the adjacent soil in this project.

The increases in wood nitrogen contents that occur during soft rot decay are primarily due to microorganisms (see section 1.3.3), thus increases in wood nitrogen contents are indicative of the microbial biomass in the wood. Wood nitrogen contents have been

monitored in soil burial studies on this basis (Mowe, 1983; Briscoe, 1987; Nayagam, 1987). Mowe (1983 *op cit*) attempted to monitor changes in the microbial population of soil adjacent to and at a distance from decaying and non-decaying wood blocks by determining the nitrogen contents of soil samples collected from these areas. However, no significant differences in the nitrogen contents of any group of soil samples was found and the measurement of the soil nitrogen content was not considered for use in the current work. Nitrogen contents of the wood blocks were measured in the work reported here, both as an indicator of the microbial colonisation of buried wood blocks and to determine the fate of the additional nitrogen present in ammonia and ACA-treated wood blocks. The nitrogen as ammonia contents of the ammonia and ACA-treated wood blocks were also measured using the technique developed by Briscoe (1987 *op cit*).

1.5.3 Determining the preservative metal contents of wood and soil.

Some loss of preservative metals can occur from preservative treated wood to adjacent soil and this can influence the soil microbial population (see section 1.4.3). In the current work the preservative metal content of soil samples and wood blocks were measured, so that metal losses from treated wood blocks and their accumulation in adjacent soil could be measured.

BS 5666: Part 3: 1979 describes a procedure for determining the copper, chromium and arsenic content of treated wood. Samples are leached with a mixture of dilute sulphuric acid (2.5M) and hydrogen peroxide solution (100 volumes) and the metal content of the resulting solution is determined either colourimetrically or by atomic absorption spectrophotometry. However, where the nitrogen

content of a wood sample is to be determined, the preservative metal content of the same sample can also be measured using the technique reported by King *et al* (1981b). The wood sample is completely digested using concentrated sulphuric acid (18M) and hydrogen peroxide solution (100 vols.). After the nitrogen content of the solution has been measured, its preservative metal content is determined by atomic absorption spectrophotometry. Since the nitrogen content of wood samples was to be measured during the current work, the preservative metal content of the wood was determined by the latter technique.

A number of techniques are available to measure the copper, chromium and arsenic content of soil, and these have been reviewed by Hesse (1971). However, no single technique is reported which would allow the determination of concentrations of all three preservative metals using one soil sample. De Groot *et al* (1979) employed two analytical techniques in their study: one to determine the arsenic content of the soil, and the other to measure its copper and chromium concentrations. In the current work various techniques were investigated so that the measurement of copper, chromium and arsenic could be carried out on a single small (1-3g) soil sample.

1.6 Aims of the present work.

CCA and ACA are effective wood preservatives, though failures have occurred as a result of soft rot decay at the wood surface. Although extensively researched, the chemical and microbiological changes at the wood/soil interface during soft rot decay of untreated and preservative treated wood are not fully understood. The overall aim of this study was to investigate the chemical and microbiological changes at the interface between soil, and wood, which had been treated with CCA or ACA wood preservatives.

This work was divided into three experimental programmes, the aims of which are presented below.

Experimental programme 1.

Losses of preservative metals from CCA-treated wood blocks during leaching and soil burial, and the effect of pre-burial leaching on subsequent moisture uptake by wood blocks during soil burial.

The aims of this experimental programme were as follows:

1. to quantify losses of copper, chromium and arsenic from CCA-treated blocks during,

   i. cold water (20±2°C) leaching, and

   ii. soil burial, and thus investigate any relationship(s) between these losses,

2. to quantify any accumulation of copper, chromium and arsenic in soil adjacent to buried, CCA-treated blocks,

3. to investigate the effect of pre-burial leaching on subsequent moisture uptake by wood blocks during soil burial.

Experimental programme 2.

The effect of CCA on microbial activity in the wood-soil system.

The aims of this programme were as follows:

1. to determine the effect of untreated and CCA-treated wood on microbial activity in adjacent soil,

2. to investigate changes in the microbial activity in buried, decaying wood blocks,

3. to determine the effect of CCA on microbial activity in buried wood blocks.

Experimental programme 3.

Chemical and microbiological studies on the effect of ammonia and ACA on the wood-soil system.

The aims of this programme were as follows:

1. to determine the effect of the increased nitrogen content of ammonia and ACA-treated wood blocks on,

   i. the microbial activity in adjacent soil,

   ii. the microbial activity in buried wood blocks,

   iii. the weight loss of the wood blocks, and

   iv. the moisture content of the wood blocks,

2. to quantify losses of copper, arsenic and nitrogen from ACA-treated blocks during,

   i. cold water leaching, and

   ii. soil burial,

3. to quantify any accumulation of copper and arsenic in soil adjacent to buried, ACA-treated blocks,

4. to determine the effect of ACA-treated wood on the microbial

activity in adjacent soil,

5. to determine the effect of ACA on microbial activity in buried wood blocks.

As a result of these measurements it was hoped to compare the performances of the two preservatives, with particular regard to the increased nitrogen contents of the ACA-treated wood.

CHAPTER 2

MATERIALS AND METHODS

MATERIALS AND METHODS

2.1 Introduction.

Many of the methods used in the three experimental programmes of this project were common to them all. Hence, accounts of the experimental programmes and the methods employed are presented solely in this chapter under the appropriate headings.

An outline of each experimental programme is presented detailing the measurements carried out, the techniques employed, the number of replicate blocks and the sampling times, and this is followed by a description of the methods used. A summary of the methods used in each experimental programme is given at the end of this chapter (table 2.4).

Three wood species were chosen for experiments undertaken during the current work; the softwoods Scots pine (*Pinus sylvestris*, L.) and Sitka spruce (*Picea sitchensis*, Carr) and the hardwood lime (*Tilia vulgaris*, Hayne). All three have previously been used experimentally at this laboratory (Mowe, 1983; Briscoe, 1987; Nayagam, 1987). Of the two softwoods Scots pine is considered to be relatively easy to treat, while Sitka spruce is regarded as a refractory wood species (see section 1.2.3).

2.1.1 Outline of experimental programme 1.

Losses of preservative metals from CCA-treated wood blocks during leaching and soil burial, and the effect of pre-burial leaching on subsequent moisture uptake by wood blocks during soil burial.

Leaching studies.

CCA leaching study 1.

12 blocks of each wood species were treated with the following levels of preservative; no preservative (control), a commercial level of CCA (3%w/v CCA) and a relatively high level of CCA (5%w/v CCA). After preservative treatment and curing 6 blocks of each wood species and treatment were leached and the concentrations of copper and chromium in the leach liquors determined. The nitrogen and preservative metal (copper, chromium and arsenic) contents of all wood blocks were also determined.

CCA leaching study 2.

For practical reasons arsenic analysis of the leach liquors was not possible when the first leaching study was carried out. Therefore a further leaching study was undertaken using 6 replicate 3 and 5%w/v CCA-treated blocks of each wood species, but including arsenic analysis of the leach liquors.

Soil burial studies.

Soil burial study 1.

6 replicate unleached, untreated and CCA-treated blocks of each wood species were buried. Leached CCA-treated and untreated pine blocks were also included. Groups of replicate blocks were sacrificed after 3, 6 and 12 weeks of soil burial. Upon uplift the moisture

content and weight loss of each block were determined. The nitrogen and preservative metal contents of the wood blocks were determined by analysis. Concentrations of copper, chromium and arsenic in soil adjacent to the buried blocks were measured using the analytical technique described later (section 2.4.1).

Soil burial study 2.

12 replicate blocks of each wood species were subjected to the three treatments (untreated, 3 and 5%w/v CCA-treated), after which half were leached. Groups of replicate blocks were sacrificed after 6, 12, 18 and 36 weeks of soil burial and the moisture content and weight loss of each block were determined.

The nitrogen contents of unleached and leached 3%w/v CCA-treated blocks were determined after 18 and 36 weeks of burial; unburied 3%w/v CCA-treated lime blocks were similarly analysed.

2.1.2 Outline of experimental programme 2.

The effect of CCA on microbial activity in the wood-soil system.

This experimental programme consisted of a single soil burial study. For each wood species the treatments were as follows,

i. untreated.

ii. a sub-toxic level of CCA (0.25%w/v for the softwoods and

0.5%w/v in the case of lime).

iii. a toxic level of CCA (5%w/v for all wood types).

Replicate sets of blocks were prepared for the sampling intervals indicated in table 2.1. A replicate set of untreated lime blocks was not prepared for the final time interval, since it was felt that they would be too heavily decayed at this stage to yield any useful data. Some sampling times were chosen to coincide with the time at which measurable decay was considered likely to begin in those blocks which were expected to decay. These additional sampling times are indicated in table 2.1. In order to keep the total number of samples within manageable limits, only blocks in which decay was considered likely to be beginning were prepared for uplift at these additional sampling times.

Due to the number of wood block analyses involved 4 replicate blocks of each wood species, treatment and sampling interval were prepared. 6 replicate blocks had been used in the past, though 4 replicates is the minimum recommended in BS 6009: 1982.

Table 2.1 Sampling intervals (X) selected for each wood species and treatment (experimental programme 2).

Wood species	Wood treatment	Sampling interval (weeks)								
		0	2	3	4	6	9	12	18	24
Lime	Untreated	X	X	X	X	X		X		
	0.5%w/v CCA	X		X		X	X	X		X
	5%w/v CCA	X		X		X		X		X
Pine/spruce	Untreated	X		X		X	X	X		X
	0.25%w/v CCA	X		X		X		X	X	X
	5%w/v CCA	X		X		X		X		X

At each sampling interval, the buried wood blocks were uplifted and their adjacent soil collected. The moisture content and level of dehydrogenase activity of the soil collected from adjacent to the buried wood blocks and of samples taken at some distance from the

buried blocks was measured. On uplift the buried wood blocks were split into portions and immediately assayed for dehydrogenase activity in the outer wood surface (immediately in contact with the soil) and the inner portion of the wood. The moisture content and weight loss of the wood blocks were also determined. The oven-dried portion of the buried wood block was used for wood nitrogen content determinations. The nitrogen content of the unburied blocks was also determined.

2.1.3 Outline of experimental programme 3.

Chemical and microbiological studies on the effect of ammonia and ACA on the wood-soil system.

A cold water (20 ± 2°C) leach study and a soil burial study were included in this experimental programme.

For the cold water leach study 6 blocks of each wood species were treated with a 1.41%w/v ACA solution. After the curing and fixation period, the blocks were subjected to a cold water leach. The leach liquors and wood blocks were analysed for levels of nitrogen, copper and arsenic. Background levels of copper and arsenic present in the leach liquors were measured using distilled water samples.

For the soil burial programme 4 replicate blocks were prepared for each sampling interval (table 2.2) and wood treatment. The following wood treatments were utilised,

i. untreated.

ii. an ammonia solution.

ii. a sub-toxic level of ACA (0.07%w/v for the softwoods and 0.14%w/v in the case of lime; copper content equivalent to 0.25 and 0.5%w/v CCA respectively).

iv. a toxic level of ACA (1.41%w/v for all wood types; copper content equivalent to 5%w/v CCA).

Table 2.2 Sampling intervals (X) selected for each wood species and treatment (experimental programme 3).

Wood species	Wood treatment	Sampling interval (weeks)						
		0	1	2	3	6	12	18
Lime	Untreated	X	X	X	X	X	X	X
	Ammonia	X	X	X	X	X	X	
	0.14%w/v ACA	X	X	X	X	X	X	X
	1.41%w/v ACA	X	X	X	X	X	X	X
Pine/spruce	Untreated	X			X	X	X	X
	Ammonia	X			X	X	X	X
	0.07%w/v ACA	X			X	X	X	X
	1.41%w/v ACA	X			X	X	X	X

Samples of unburied blocks were analysed for levels of nitrogen, nitrogen as ammonia, copper and arsenic. These analyses were carried out on undried samples so that all nitrogen present in the ammonia and ACA-treated wood would be detected. Concentrations determined were corrected for block moisture content, which had been measured using a further sample of the same block.

On block uplift the dehydrogenase activity and moisture content of the soil adjacent to the buried wood blocks and soil at a distance from any block were immediately measured. The dried soil samples from around the buried blocks were stored, and their copper and arsenic contents subsequently determined.

On exhumation of the wood blocks the dehydrogenase activity in the outer wood layer and the inner wood were determined, along with the block moisture contents and weight losses. The oven-dried wood was used for nitrogen content determinations at every sampling interval. The nitrogen as ammonia contents of all softwoods sampled after 3 and 6 weeks of burial were measured, as were the levels in

lime blocks after 1, 2 and 3 weeks of burial. The copper and arsenic contents of all untreated and ACA-treated blocks were determined in blocks buried for 18 weeks.

METHODS

Distilled or de-ionised water and grade 'A' glassware were used. Analar reagents were used where necessary, for instance, in the case of the determination of the nitrogen content of wood blocks the use of nitrogen-free, and therefore analar, hydrogen peroxide and concentrated sulphuric acid was essential. However, where the use of analar reagents was not a requirement, for instance in the case of the methanol used in the dehydrogenase assays, standard laboratory grade reagents were used.

2.2 Preparation of wood blocks.

Freshly felled, bolts of pine and spruce were obtained from a local forest at Dunkeld by courtesy of the Forestry Commission. A bolt of freshly felled lime from a local source was provided by Mr E. Hamilton (forester) of City of Dundee District Council Parks Department. All bolts were reduced to quartersawn planks and stored in a deep freeze at -18°C until required. When needed the planks were thawed and dried in a fan oven at 40°C for a minimum period of 2 weeks. Radial, evaporative surfaces were removed to a depth of at least 2mm and blocks measuring 30*20*5mm were then cut from the sapwood (rings 3-25 measured from the cambium) of the planks (figure 2.1). The 20*5mm faces of these blocks were in transverse section, while the 30*5mm and 30*20mm faces were in tangential and radial faces respectively. These blocks are larger than the 10*10*5mm blocks

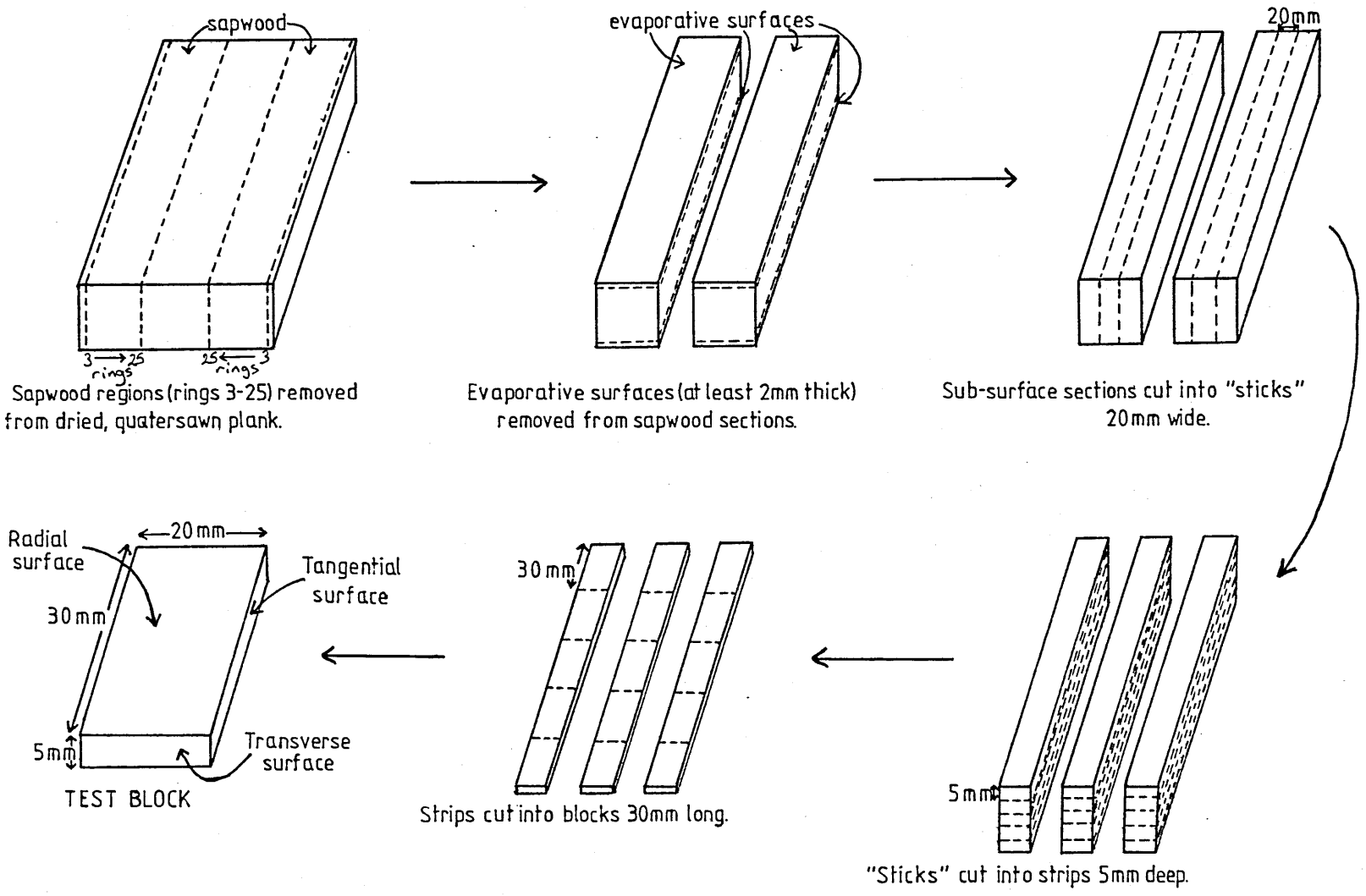


Figure 2.1 Conversion of dried planks to wood blocks.

generally employed at this laboratory (Briscoe, 1987; Nayagam, 1987). The larger blocks had previously been employed by Mowe (1983) and are used because they have a large surface area which results, on burial, in a greater wood-soil interface. Once cut the blocks of each species were placed in plastic bags for storage in the laboratory.

Prepared sapwood blocks of each species were randomly selected from their storage bags, numbered, dried in an oven at 102±2°C to constant weight and their initial dry weights, determined to the nearest 0.0001g, recorded prior to use.

2.2.1 Preparation of preservative solutions.

Copper chrome arsenate (CCA) solution.

Solutions of CCA type 2 (BS 4072: 1974) were prepared, comprising potassium dichromate ($K_2Cr_2O_7$), 45%; copper sulphate ($CuSO_4.5H_2O$), 35%; and arsenic pentoxide dihydrate ($As_2O_5.2H_2O$), 20%, except that arsenic pentoxide pentahydrate ($As_2O_5.5H_2O$) was used in preference to the dihydrate specified in the standard, since the pentahydrate is more readily dissolved.

Ammoniacal copper arsenate (ACA) solution.

A solution of 1.41%w/v ACA was prepared (Hulme and Butcher, 1977c), with arsenic pentoxide pentahydrate ($As_2O_5.5H_2O$) again being used in preference to the dihydrate. 14.168g of $As_2O_5.5H_2O$ was dissolved in a mixture of 100cm^3 ammonia solution (specific gravity 0.88) and approximately 300cm^3 distilled water. 16.484g of basic copper carbonate ($CuCO_3.Cu(OH)_2.H_2O$) was added and the mixture stirred until it was dissolved, at which point the solution was made up to 2 litres in a volumetric flask using distilled water. This solution contained 1.35%w/v ammonia and 4.45g per litre copper (equivalent to the copper content of a 5%w/v CCA solution).

A 1.35%w/v ammonia solution was prepared by making 100cm^3 0.880 ammonia solution up to 2 litres in a volumetric flask using distilled water. This solution was used to make 1 in 20 and 1 in 10 dilutions of the 1.41%w/v ACA solution, giving solutions of 0.07 and 0.14%w/v ACA respectively. The 1.35%w/v ammonia solution was also used to treat blocks in experimental programme 3.

The concentrations of the preservative metals in all CCA and ACA preservative solutions were checked by chemical analysis using atomic absorption spectrophotometry (AAS) with a standard additions method (section 2.5.4). The nitrogen contents of the ammonia and ACA solutions were determined using the Kjeldahl method (section 2.5.3). The pH of all treating solutions was measured using a calibrated pH meter (Corning pH meter, model 7). The density of the solutions was determined by accurately weighing known volumes of each solution. The results of all of these determinations are presented in Appendix 1.

2.2.2 Treatment of wood blocks with preservative.

Blocks were impregnated with preservative solution according to BS 6009 (1982), except that blocks were left submerged in the solution for 30 minutes, rather than for 2 hours, to avoid the loss of soluble nutrients (King *et al*, 1981b).

The fully saturated blocks were removed from the preservative solution one by one, and excess liquid was removed by light blotting with absorbent paper. All blocks were immediately weighed to the nearest 0.0001g. The preservative impregnated blocks were placed on glass sheets (30*5mm faces down) in a glass desiccator from which the desiccant had been removed. A small amount of water was placed in the base of the desiccator and a beaker containing about 5cm^3 of xylene was also included. The desiccator was then kept sealed for two weeks.

For the CCA-treated blocks, the desiccator was gradually opened in the course of the third week and left fully open for a fourth week. ACA-treated blocks were removed from the desiccator after 2 weeks in the moist, enclosed environment and placed in a fan oven (25°C) for five weeks according to the method of Briscoe (1987), after which time he had determined the nitrogen content of these blocks to be approximately constant. During their curing and fixation period (4 weeks for CCA-treated blocks and 7 for ACA-treated blocks) blocks were turned onto their opposite face twice weekly.

Liquid uptake was calculated by deducting the initial dry weight of the blocks from their weight immediately after impregnation. These weights were converted to a volume measurement by dividing them by the density of the appropriate solution. Using the concentrations of the preservative metals in each treating solution (see Appendix 1) and the volume of liquid taken up by each block, the preservative metal content of each block was calculated and expressed as a %w/w.

2.2.3 Leaching procedure.

The leaching regime employed was based on that previously used in this laboratory (Briscoe, 1987), adapted for the leaching of the larger sized blocks being used here. 3 blocks of the same wood species and treatment were placed in the bottom of a 1 litre beaker and weighted down with glass slides. A vacuum was drawn for 15 minutes, after which 600cm^3 of water was introduced and any residual vacuum released. In leaching experiment 1, experimental programme 1 deionised water was used, though for the two subsequent leaching experiments distilled water was available and this was employed. After the blocks were impregnated with water, the weights were removed, the container covered and placed on a magnetic stirrer. The

leach liquor was changed every 24 hours. If the metal content of the leach liquor was to be measured it was reserved at this point.

Analysis of the leach liquors of the first CCA cold water leaching experiment indicated that after 5 days the blocks were losing only minimal quantities of copper and chromium (section 3.1.1.3) and so the leaching procedure was terminated at this point. Blocks were removed from the water and placed in partially covered petri dishes at 25°C until a constant weight was achieved.

2.2.4 Analysis of the leach liquors.

For experimental programmes 1 and 3 duplicate sets of daily leach liquors for each wood species and preservative treatment were analysed for preservative metal concentrations. The individual daily leach liquors were acidified with $1cm^3$ of concentrated sulphuric acid (18M) and then placed on a hot plate. The solution was evaporated down to approximately $75cm^3$ and, when cool, filtered through Whatman 541 into a $100cm^3$ volumetric flask and made up to the mark. Concentrations of the preservative metals were measured using the AAS and a standard additions method (section 2,5,4). Three $600cm^3$ distilled water samples were concentrated as described for the leach liquors and the concentrations of copper, chromium and arsenic determined.

The nitrogen content of the leach liquors was determined as described for the wood blocks (section 2.5.3) and the ammonia and ACA solutions, with $5cm^3$ of the acidified leach liquor being introduced into the Markham still, in place of the digested wood sample.

2.3 Soil burial.

2.3.1 Preparation of soil.

Top soil, classified as a sandy loam, was collected from the Scottish Crop Research Institute, Invergowrie, Tayside. It had received no biocide treatment for at least one year prior to its collection. The soil was sieved through a 2mm stainless steel screen and then stored in large plastic bins covered with loose fitting lids until required. For the first 3 burial experiments sieving was done by hand, though for experimental programme 3 a mechanical sieve was used. For either hand or mechanical sieving to be carried out, air drying of the soil was necessary. In the former case soil was dried to a moisture content of about 10-19%w/w prior to sieving, though in the latter case further air drying was required, so that the moisture content of the soil was less than 10%w/w. It has been demonstrated (Bravery, 1968a) that air drying soil does not reduce the wood decaying potential of the natural soil microflora, and may even enhance it.

The pH of the soil was measured (BS 1377: 1975) using three replicate soil samples. The moisture content and water holding capacity of the soil were determined (Savory and Carey, 1973) immediately prior to its use in each soil burial study. Results of the pH, moisture content and water holding capacity determinations are presented in Appendix 2.

2.3.2 Soil burial procedure.

Blocks which were to be sacrificed at the same sampling interval in each soil burial study, were placed in plastic bags, different bags being used for the softwood and hardwood blocks. For experimental programmes 2 and 3, some potential block positions were to be left unfilled, so that soil samples at a distance from any buried wood blocks, but at the same depth as the blocks, could be collected. These samples were required for the determination of background soil dehydrogenase activity and additional blocks, representing the background soil samples, were included in the plastic bags where necessary. Templates were cut to the size of the boxes to be used and block positions were marked, with a minimum distance of 30mm between each block. Wood blocks were removed from the plastic bags at random and the templates marked with either the number of the block withdrawn or as a background soil sample.

Plastic boxes (16.5cm wide * 24.5cm long * 10.5cm deep) were filled to a depth of 55mm with the prepared soil. Blocks were positioned using the prepared template and inserted until the upper 20*5mm face was level with the soil surface. Hardwood and softwood blocks were buried separately, and different boxes were used for each sampling interval. Boxes were filled to a depth of 80mm giving 25mm of soil below the lower horizontal face of the block and 25mm of soil above its upper face. Water was added evenly over the soil surface to bring the soil to 80% (for lime) and 100% (for pine and spruce) of its water holding capacity.

Boxes were partially covered and placed in the dark in a room with a constant temperature of 25°C. The soil was maintained at the

required water holding capacity by weighing the boxes twice weekly and, where necessary, sprinkling distilled water over the soil surface.

2.3.3 Sampling.

Different techniques were employed for the recovery of buried wood blocks, depending on whether the wood block alone was to be sampled (soil burial 2, experimental programme 1), or whether the adjacent soil was also to be recovered (experimental programmes 2, 3 and 1, soil burial 1).

Wood blocks only recovered.

The template was positioned on the soil surface and buried blocks were located using forceps and recovered. Any adhering soil was gently brushed from the block, which was immediately weighed, dried to constant weight at 102±2°C, cooled in a desiccator and reweighed.

Wood block and adjacent soil recovered.

The upper 25mm of the soil was carefully removed, exposing the upper 20*5mm surface of the block. Blocks were recovered together with soil surrounding their vertical faces using an 'extractor'. The 'extractor' consisted of two 'U'-shaped pieces of metal (see figure 2.2), with an internal cross-section such that a layer of soil less than 2mm thick was collected along with the block. The soil within the 'extractor' and any adhering to the block was removed by gentle brushing and collected in a Pyrex petri dish. Compressed soil below the block was not used.

In soil burial 1, experimental programme 1, the collected soil was dried at 102±2°C, placed in resealable plastic bags and stored for preservative metal analysis. For experimental programmes 2 and 3, each soil sample was gently mixed and an approximately 1.5g (fresh

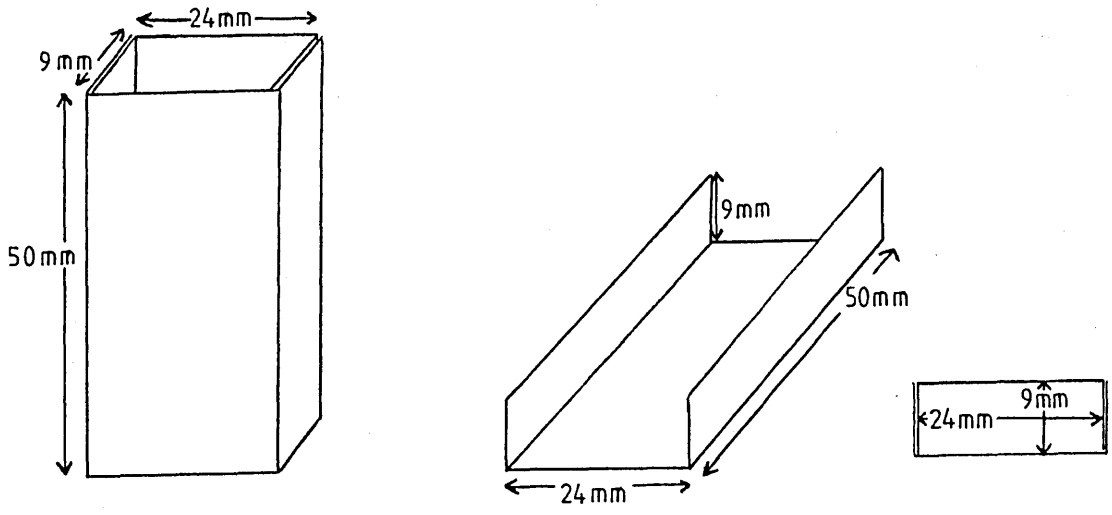


Figure 2.2 Diagram of block and soil extractor.

weight) portion accurately weighed. This sample was placed in a sterile, screw-topped test tube (120*15mm), which already contained 15mg calcium carbonate, for the determination of the dehydrogenase activity of the soil (section 2.4.2). Any remaining soil was accurately weighed, dried in an oven at 102±2°C and its moisture content determined. The moisture content values were used to express the dehydrogenase data obtained on a dry weight basis.

In soil burial 1, experimental programme 1, the uplifted block was weighed, dried to constant weight at 102±2°C and reweighed. For experimental programmes 2 and 3 the uplifted block was weighed, placed on a glass sheet and split in half along the grain with the aid of a Stanley knife (figure 2.3). One half of the block was then weighed, dried to constant weight at 102±2°C and reweighed to determine its moisture content. The final dry weight of the total wood block was then derived from the wet weight of the total block and the moisture content of the half block and used to determine the weight loss of the total block. The outer surface of the remaining half of the block, which had been in contact with the soil, was removed using the Stanley knife, finely divided, its fresh weight accurately determined (generally about 0.1 - 0.5g) and then placed in a screw-top test tube for the dehydrogenase assay. The inner wood (approximately 0.5-1.6g) was finely divided and approximately 0.1 - 0.5g was weighed and placed in a screw-top test tube for dehydrogenase analysis. The remaining inner wood was weighed, dried at 102±2°C and its moisture content calculated. This moisture content value was employed to express the outer and inner wood dehydrogenase data on a dry weight basis. The second moisture content was considered to be a more accurate reflection of the moisture level in the assayed samples than the first value, since some moisture loss occurred during handling.

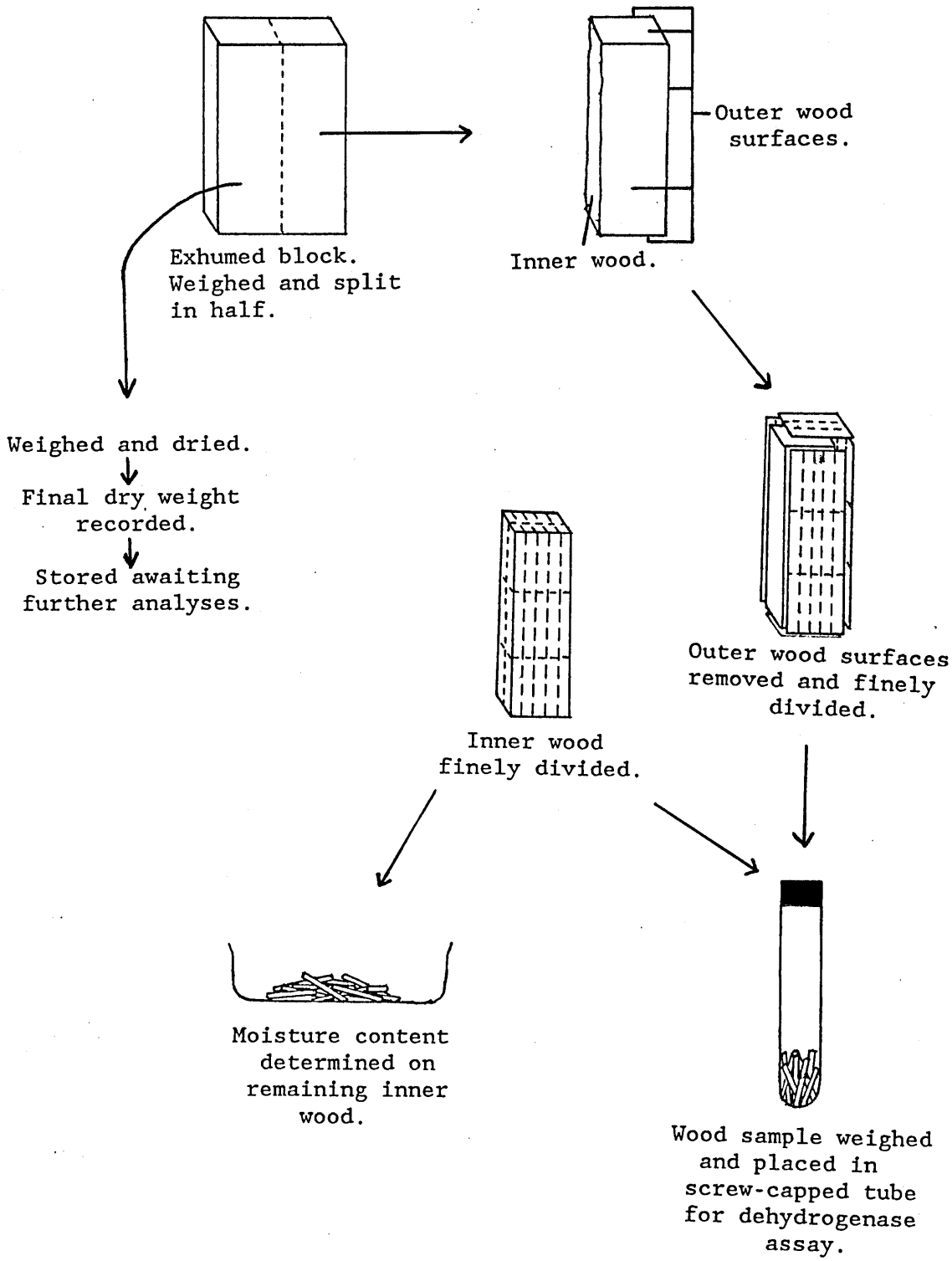


Figure 2.3 Division of exhumed wood block for dry weight measurement and dehydrogenase activity assay.

Wood samples requiring analysis for nitrogen or metal content were finely divided using a Stanley knife, placed in resealable plastic bags and stored in the laboratory awaiting analysis.

2.4 Analyses of soil.

2.4.1 Comparative study of methods for the determination of copper, chromium and arsenic levels in soil.

Introduction.

Three methods were compared for their efficiency in determining the levels of preservative metals in soil, namely,

a. using a concentrated sulphuric acid (18M) and hydrogen peroxide (100 volumes) digest, previously used in this laboratory to determine soil nitrogen levels (Mowe, 1983).

b. using a leaching method employing the chelating agent ethylenediaminetetraacetic acid (EDTA), (Viro, 1955),

i. employing the disodium salt at pH 9.

ii. employing the ammonium salt at pH 7.

iii. employing the ammonium salt at pH 9.

c. using a leaching method based on BS 5666:Part 3:1979 employing 2.5M sulphuric acid and hydrogen peroxide (100 vols.).

The efficiency of each method was assessed by determining the copper, chromium, and, in some cases, the arsenic content of :

1. top soil, classified as a sandy loam, used in all soil burial studies (see section 2.3.1).

2. top soil as described in (1.), but with a known amount of copper, chromium and arsenic added.

Preparation of samples.

Soil, previously sieved to pass a 2mm mesh, was dried in a fan oven at 40°C for a minimum period of 6 hours. The soil sample was divided into 2 approximately equal portions, of about 50g each, accurately weighed and placed in 100cm^3 beakers. A known volume of CCA solution was added to one beaker and the same volume of distilled

water was added to the other. The beakers were covered and left overnight. The soil was then dried (102±2°C), accurately weighed, ground using a pestle and mortar and stored to await analysis.

The residual moisture content of the soil after the initial drying period was calculated using the weight of the soil sample before the addition of the CCA solution/water and the weight after oven-drying. Since the metal concentration of the CCA solution was known, the level of additional copper, chromium and arsenic in the soil to which the CCA solution had been added could be estimated and was expressed in ug g^{-1} soil (dry weight). CCA was added in sufficient concentration to give additional levels of the metal elements in the range 10-120ug copper g^{-1} soil, 19-200ug chromium g^{-1} soil and 10-140ug arsenic g^{-1} soil.

Methods of analysis.

Method 1.

An oven-dried (102±2°C), accurately weighed sample (approximately 1g) was placed in a micro-Kjeldahl flask ($30cm^3$). $3cm^3$ concentrated sulphuric acid (18M) was added, followed by the dropwise addition of approximately $1cm^3$ hydrogen peroxide (100 vols.). The flasks were then heated until dense, white fumes of sulphur trioxide were observed, at which point the flasks were removed from the heat and allowed to cool for a minimum period of 2 minutes. After cooling, a further $1cm^3$ hydrogen peroxide was added to the flask, which was then returned to the heat. Heat was applied until the reappearance of the dense, white fumes.

The cooling and heating cycle was repeated, with additions of hydrogen peroxide as indicated, until no brown/grey colouration was observed when the fumes formed. At this point the solution was an off-white colour. This procedure took a minimum of 3 hours. Once cool the solution was filtered through Whatman 541 into a $100cm^3$

volumetric flask and made up to the mark. The concentration of copper, chromium and arsenic in the final solution was measured using a standard additions technique (2.5.4), and the level of each metal in the soil sample (ug g^{-1}) was then calculated.

Method 2.

A 0.05M EDTA solution was prepared using either the disodium or the ammonium salt and, in the latter case, its pH was adjusted by the addition of ammonium hydroxide solution. An oven-dried (102±2°C), accurately weighed soil sample (approximately 1g) was placed in a centrifuge tube and 10cm^3 of the EDTA solution added. The soil was suspended in the solution using a vortex mixer, and the suspension mixed frequently for a further 15 minutes, after which time it was centrifuged at *3000g for 5 minutes. The supernatant was filtered through Whatman 541 into a 100cm^3 volumetric flask. A further 5cm^3 of the EDTA solution was added to the centrifuge tube and the pellet resuspended. The mixture was mixed for 15 minutes, centrifuged and filtered into the 100cm^3 volumetric flask as previously described. The solution was then made up to the mark.

The copper and chromium concentration of the final solution were measured using a standard additions technique (section 2.5.4), and the level of each metal in a gramme of soil (ug g^{-1}) was calculated.

Method 3.

This method is based on BS 5666:Part 3 (1979), modified for use with the smaller sample available for analysis. The amount of hydrogen peroxide added in relation to the volume of 2.5M sulphuric acid employed has been reduced and the temperature used increased.

An oven-dried (102±2°C), accurately weighed sample (approximately 3g) was placed in a 100cm conical flask. 25cm^3 of 2.5M sulphuric acid was added, followed by 1cm^3 of hydrogen peroxide. The flask was heated on a hotplate in a fume cupboard at approximately 100°C for 30

minutes. After heating, the flask was removed from the hotplate, approximately 20cm^3 of distilled water added, and left to cool.

The solution was poured into a centrifuge tube (28*95mm) and 3 rinsings of the flask were added. The solution was centrifuged at *3000g for 5 minutes, filtered through Whatman 541 filter paper into a 100cm^3 volumetric flask, and then made up to the mark. Aliquots of the solution were analysed for preservative metal levels by a standard additions technique (section 2.5.4) and the amount of each metal in the soil sample (ug g^{-1}) was calculated.

In all cases reagent blanks were analysed for preservative metals levels.

2.4.2 Measurement of dehydrogenase levels in soil.

The dehydrogenase method used was that of Casida *et al* (1964), modified for use with a small soil sample (Mowe, 1983). The weighed sample, which had been placed in a screw-topped test tube containing 15mg calcium carbonate (section 2.3.3), was saturated with 2cm^3 0.75%w/v 2,3,5-triphenyltetrazolium chloride (TTC) aqueous solution. The tubes were sealed and mixed thoroughly, so that the calcium carbonate was evenly distributed. Care was taken to ensure that, at the conclusion of the mixing, the bulk of the sample was beneath the surface of the solution. Tubes were incubated in the dark for 24 hours at 30°C. A reagent blank consisting of 15mg calcium carbonate and 2cm^3 0.75%w/v TTC aqueous solution was also incubated.

Triphenyltetrazoliumformazan (TTF) was extracted from the assayed soil sample using a total of 8cm^3 of methanol. In experimental programme 2 the soil was rinsed with 2 portions of methanol (solvent extraction regime I), as recommended by Mowe (1983 *op cit*). However, it was felt that not all the TTF formed was being removed from the

assayed wood and soil samples by this method, as some red colour could be seen around the wood samples after the extraction was complete. Therefore, a second solvent extraction regime using 8 portions of methanol was employed in experimental programme 3. The two regimes are described below.

Solvent extraction regime I.

The mixture was shaken with $5cm^3$ methanol and, after allowing the larger soil particles to settle, the liquid was poured into a screw-top centrifuge tube (1.5*10cm). The remaining soil solids were rinsed with a further $3cm^3$ of methanol and the final $10cm^3$ was centrifuged at *3000g for 5 minutes.

Solvent extraction regime II.

The mixture was shaken with $1cm^3$ methanol and, after allowing the larger soil particles to settle, the liquid was poured into a screw-top centrifuge tube (1.5*10cm). The remaining soil solids were rinsed a further seven times using 1 cm^3 of methanol on each occasion and the final $10cm^3$ was centrifuged at *3000g for 5 minutes.

In each case, following centrifugation the supernatant was transferred to a glass cuvette and its absorbance measured spectrophotometrically at 485nm (LKB Ultrospec 4050). A scan of the absorbance of TTF was carried out using a UV-visible recording spectrophotometer (Shimadzu, UV-160). A broad peak of maximum absorbance, from about 478-487nm, was obtained, with a peak value of 479.8nm. The absorbance value of 485nm, recommended by previous workers (Casida *et al* 1964, *op cit*), was used in determinations, and, since it lay within the broad peak section, this did not seem unreasonable.

A standard curve of the product (TTF) in methanol was constructed in the following manner:

A stock solution of 0.333umol cm^3 TTF in methanol was prepared

by dissolving 0.01g of TTF in 100cm^3 of methanol. The following dilutions were then made up,

1. 2cm^3 stock + 4cm^3 methanol, concentration = 0.111umol cm^{-3}
2. 1cm^3 stock + 5cm^3 methanol, concentration = 0.055umol cm^{-3}
3. 1cm^3 dilution 1 + 3cm^3 methanol, concentration = 0.028umol cm^{-3}
4. 1cm^3 dilution 2 + 3cm^3 methanol, concentration = 0.014umol cm^{-3}
5. 1cm^3 dilution 3 + 3cm^3 methanol, concentration = 0.007umol cm^{-3}
6. 1cm^3 dilution 4 + 3cm^3 methanol, concentration = 0.003umol cm^{-3}

The absorbance of each solution was measured at 485nm with a blank of pure methanol. The values obtained were plotted against their corresponding concentration values and a straight line obtained, an example of which is given in figure 2.4 . Using the Minitab statistical package (Copyright - Minitab, Inc.) on the Vax-system the regression equation of,

concentration = a + b*absorbance,

was calculated. A calibration curve was drawn and an equation calculated each time the absorbance of the experimental solutions was measured.

Using the calibration equation obtained and the absorbance of each experimental solution, the concentration of TTF (umol cm^{-3}) in each solution was calculated. The weight of soil assayed was corrected to a dry weight using the moisture content determined on sampling, after which the level of dehydrogenase activity, expressed in umols product formed (TTF) g^{-1} min^{-1} (as recommended by Bolton *et al*, 1985), was calculated using the equation,

$$\text{umolTTF } g^{-1} \text{ min}^{-1} = \frac{(\text{umol } cm^{-3})*10}{[\text{weight of soil (dry)}] * (24*60)}$$

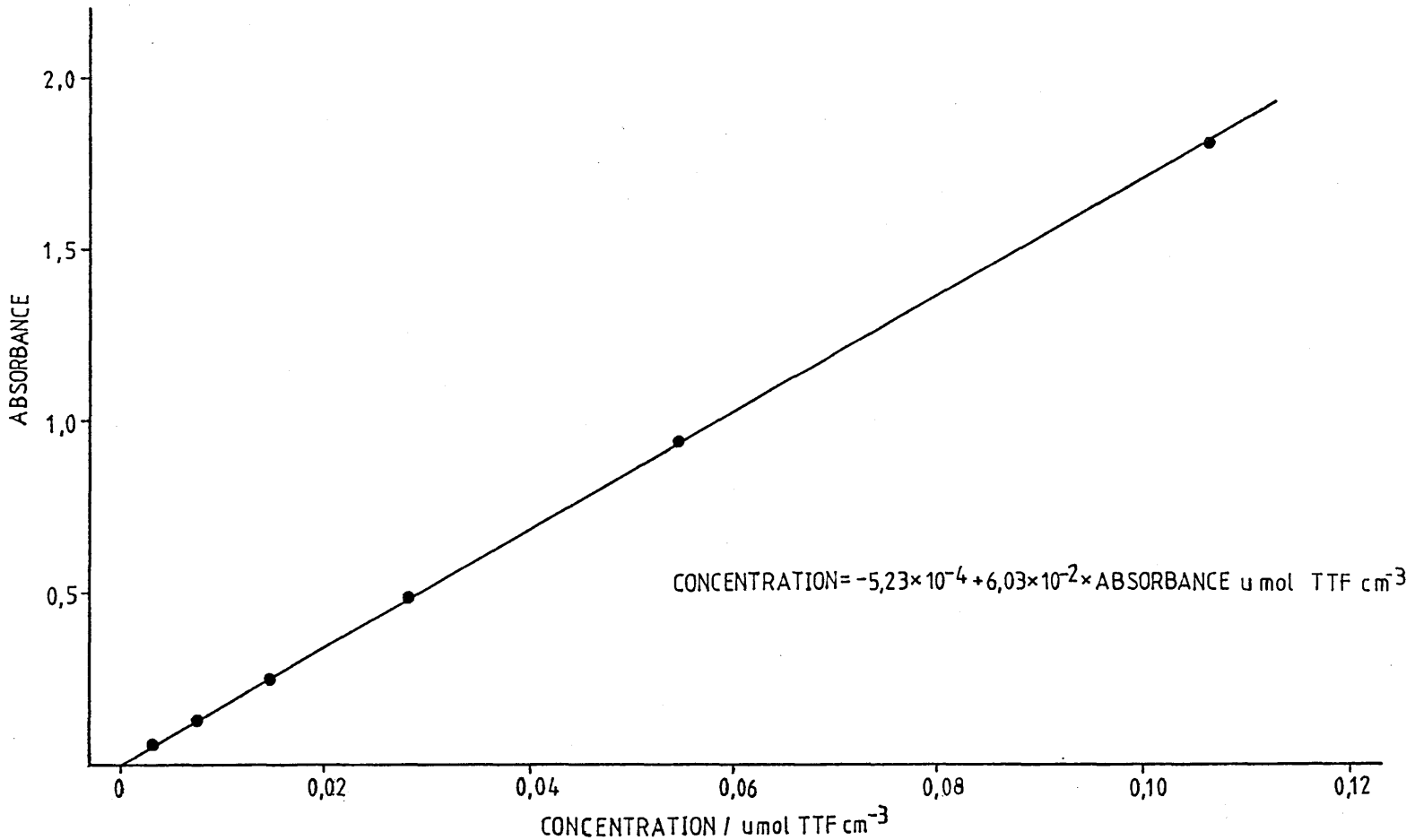


Figure 2.4 Example of a calibration curve used to calculate the concentration of TTF in test solutions.

2.5 Analyses of test blocks.

2.5.1 Calculation of moisture contents.

The moisture contents of the buried wood blocks and the soil samples were expressed on the basis of their final dry weights using the equation,

$$\text{moisture content (\%w/w)} = \frac{\text{wet weight - final dry weight}}{\text{final dry weight}} * 100$$

2.5.2 Calculation of weight losses.

The weight loss of the buried wood blocks was expressed on the basis of their initial dry weight, corrected, where appropriate, for preservative salt content. No correction was carried out for the additional nitrogen content of the ammonia and ACA-treated blocks. The dry weight of salt added to the preservative treated block during impregnation was calculated from the volume of preservative solution known to have been taken up by the block and was then added to the initial dry weight of the block.

The equation used to calculate weight loss was,

$$\text{weight loss (\%)} = \frac{(A + B - C)}{(A + B)} * 100$$

where, A = initial dry weight of the wood block (g)
B = dry weight of added salt (g)
C = final dry weight of the wood block (g)

2.5.3 Determination of the nitrogen contents.

The nitrogen content was determined using a micro-Kjeldahl digestion technique employing nitrogen-free, concentrated sulphuric acid (18M) and hydrogen peroxide (100 vols.) followed by ammonia

distillation in a Markham unit, as reported in King *et al* (1981b). On conclusion of the ammonia distillation, the sample solution was recovered from the unit and the metal content of the test samples could then be determined.

Digestion of the blocks.

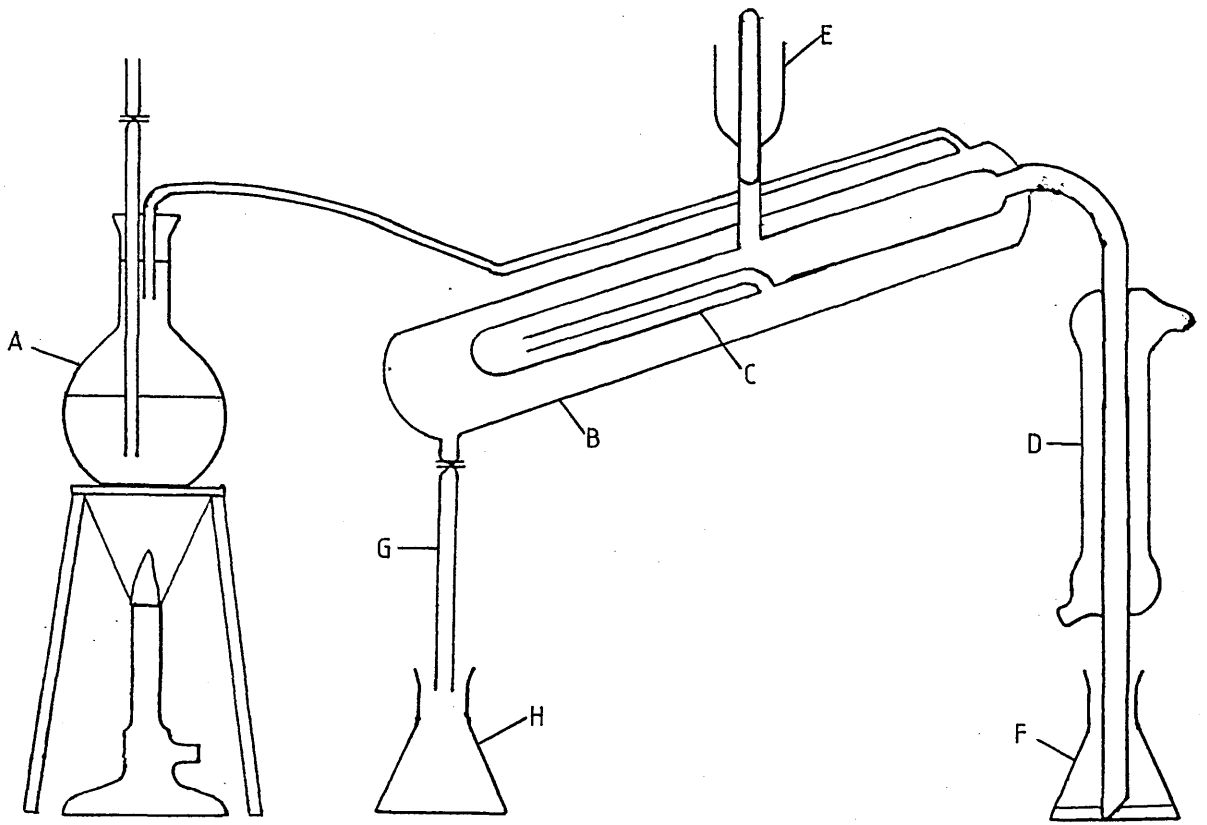
A portion of each wood sample (approximately 0.1-0.3g, dry weight) was randomly collected, dried to constant weight and accurately weighed. It was then placed in a dry micro-Kjeldahl digestion flask ($30cm^3$) and $2cm^3$ of concentrated sulphuric acid (18M) was added followed by the dropwise addition of $1cm^3$ hydrogen peroxide (100 vols.). The flasks were heated until the first signs of dense white fumes of sulphur trioxide appeared, when they were removed from the heat and allowed to cool for at least 2 minutes, prior to the addition of a further $1cm^3$ of hydrogen peroxide (100 vols.). The flasks were then returned to the heat, which was applied until the dense white fumes were again observed.

The cooling and heating cycle was repeated, with additions of hydrogen peroxide as indicated, until the wood samples were completely dissolved and the solutions remained clear and free from any brown/grey colouration when the white fumes formed. The time required to achieve this varied, with heavily decayed wood samples dissolving rapidly, while treated blocks took very much longer, on occasions in excess of 2 hours.

Once the samples were completely digested, a further $2cm^3$ of hydrogen peroxide was added to each flask and the mixture was boiled for about 2 minutes to drive off excess oxygen from the samples, which were then allowed to cool.

Nitrogen determination.

A diagram of the Markham unit used in the nitrogen determination is presented in figure 2.5.



A = 2 litre flask.

B = Outer jacket.

C = Inner jacket.

D = Condenser.

E = Funnel.

F = 100 cm^3 flask + 5cm^3 2% boric acid & 10 drops Kjeldahl indicator.

G = tubing for removal of residual solution.

H = 100cm^3 conical flask + 6 drops Kjeldahl indicator.

Figure 2.5 Markham unit used in nitrogen determinations.

Water in a 2 litre flask (A) was brought to a rapid boil and the steam generated passed in through the outer jacket (B), then into the inner jacket (C) and finally out through the condenser (D). Before starting a set of analyses, the apparatus was steamed for 10 minutes to remove any residues from previous analyses.

Digestion solutions were carefully added to funnel E and the micro-Kjeldahl flask rinsed twice with distilled water. The rinsings were also added to the funnel. The solution was slowly introduced into the inner jacket, as were two rinsings of the funnel. A $100cm^3$ conical flask (F) containing $5cm^3$ of a 2%w/v boric acid solution and 10 drops of Kjeldahl indicator (0.5g methyl red and 0.25g methylene blue dissolved in $500cm^3$ of ethanol) was placed under the condenser. $12cm^3$ of 40%w/v sodium hydroxide solution was poured into the funnel and added to the sample very slowly. A small amount of the sodium hydroxide solution was left in the funnel to prevent any loss of the gaseous ammonia. Approximately $20cm^3$ of distillate was collected, giving $25cm^3$ of solution in total, at which point the conical flask was removed from beneath the condenser.

During the analyses, some steam condensed on the inner surface of the outer jacket. This was removed via tube G and discarded. The bunsen burner was removed from beneath the boiling water, creating a pressure fall, which drew the sample from the inner to the outer jacket of the Markham still. The residual solution was collected via tube G in a $100cm^3$ conical flask (H), which contained 6 drops of Kjeldahl indicator. Water was added to the funnel and used to rinse out the unit, the washings also being collected in flask H. This rinsing procedure was then repeated.

The green boric acid-ammonia solution was titrated against standard 0.01M hydrochloric acid solution ($1cm^3$ 0.01M HCl $\equiv$ 0.14mg N), the end point being the first hint of pink colour in the

solution.

The nitrogen content (%w/w) of the wood sample was calculated as follows:

$$\text{Nitrogen content (\%w/w)} = \frac{0.014 * \text{titre}(cm^3)}{\text{weight of wood analysed (g)}}$$

The %w/w nitrogen content was expressed on the basis of the original dry weight of the wood sample. Therefore the nitrogen content value obtained was corrected for the weight of any preservative salt present in the wood block and for any weight loss of the block. Thus any observed changes in the nitrogen content of the buried blocks was real, and not an artefact produced by weight loss.

The determination of nitrogen concentration using the Markham still was standardised using a 0.1% solution of analar ammonium sulphate ($[NH_4]_2\ SO_4$), which gave a titre of $3.03cm^3$ of 0.01M hydrochloric acid solution.

2.5.4 Determination of copper, chromium and arsenic contents.

The digest solutions, recovered in flask H, were re-acidified by the addition of 2.5M sulphuric acid until a colour change from yellow to pink was observed (10-$15cm^3$ of 2.5M sulphuric acid was usually required). The solutions were filtered through Whatman 541 filter paper into $100cm^3$ flasks and made up to the mark. The concentrations of copper, chromium and arsenic in these solutions were then measured on an atomic absorption spectrophotometer by a standard additions technique. Aliquots of the solutions were pipetted into each of three $25cm^3$ volumetric flasks, labelled A, B, and C. 1 and $2cm^3$ aliquots of a standard solution containing $25ug/cm^3$ of copper and chromium and $250ug/cm^3$ of arsenic were added to the flasks labelled B and C

respectively. All 3 flasks were then made up to the mark.

The solutions were analysed for the preservative metals on a Perkin Elmer 372 atomic absorption spectrophotometer (AAS). Operating conditions for the 3 elements are shown in table 2.3.

Table 2.3 Operating conditions for the analysis of copper, chromium and arsenic by atomic absorption spectrophotometry.

Element	Wavelength (nm)	Fuel	Oxidant	Flame type
Copper	324.8	Acetylene	Air	Oxidising, lean blue
Chromium	357.9	Acetylene	Air	Reducing, yellow
Arsenic	193.7	Hydrogen	Argon	Colourless

Copper, chromium and arsenic hollow cathode lamps were used for the analysis.

The sensitivity of the AAS was maximised for each element using a machine standard solution. Separate standards were prepared for each element and these contained 5ug cm^{-3} of copper, 2ug cm^{-3} of chromium and 40ug cm^{-3} of arsenic. The absorbance readings for these standards during analysis were at least 0.250, 0.100 and 0.400 for copper, chromium and arsenic respectively.

Procedure for the AAS.

The AAS was zeroed using distilled water and its sensitivity for the element being analysed checked using the appropriate machine standard. The absorbance reading for each sample was then taken using flasks A, B and C in succession. The reading noted for each flask was the average of at least 3 consecutive 3 second digital readings. The zero reading was checked with distilled water between each of these flasks. The sensitivity of the AAS was checked using the machine standard at regular intervals throughout the analyses.

The concentrations of copper and chromium in a sample without any standard solution added (flask A) was calculated as follows :

$$\text{ug cm}^{-3} \text{ of copper or chromium} = \frac{S}{\frac{(S1 - S) + (S2 - S)}{3}}$$

Where S = absorbance reading for flask A (without standard)

S1 = absorbance reading for B (including $1cm^3$ of standard solution)

S2 = absorbance reading for C (including $2cm^3$ of standard solution)

The concentration of arsenic was calculated as above, except that the value obtained was multiplied by 10 to give the concentration of arsenic in the flask.

The concentrations of the 3 metals in the original wood sample (%w/w) were calculated using the formula,

$$\%w/w = \frac{C * 2500}{[\text{volume of aliquot}(cm^3)] * [\text{weight of wood sample (g)}]} * 100$$

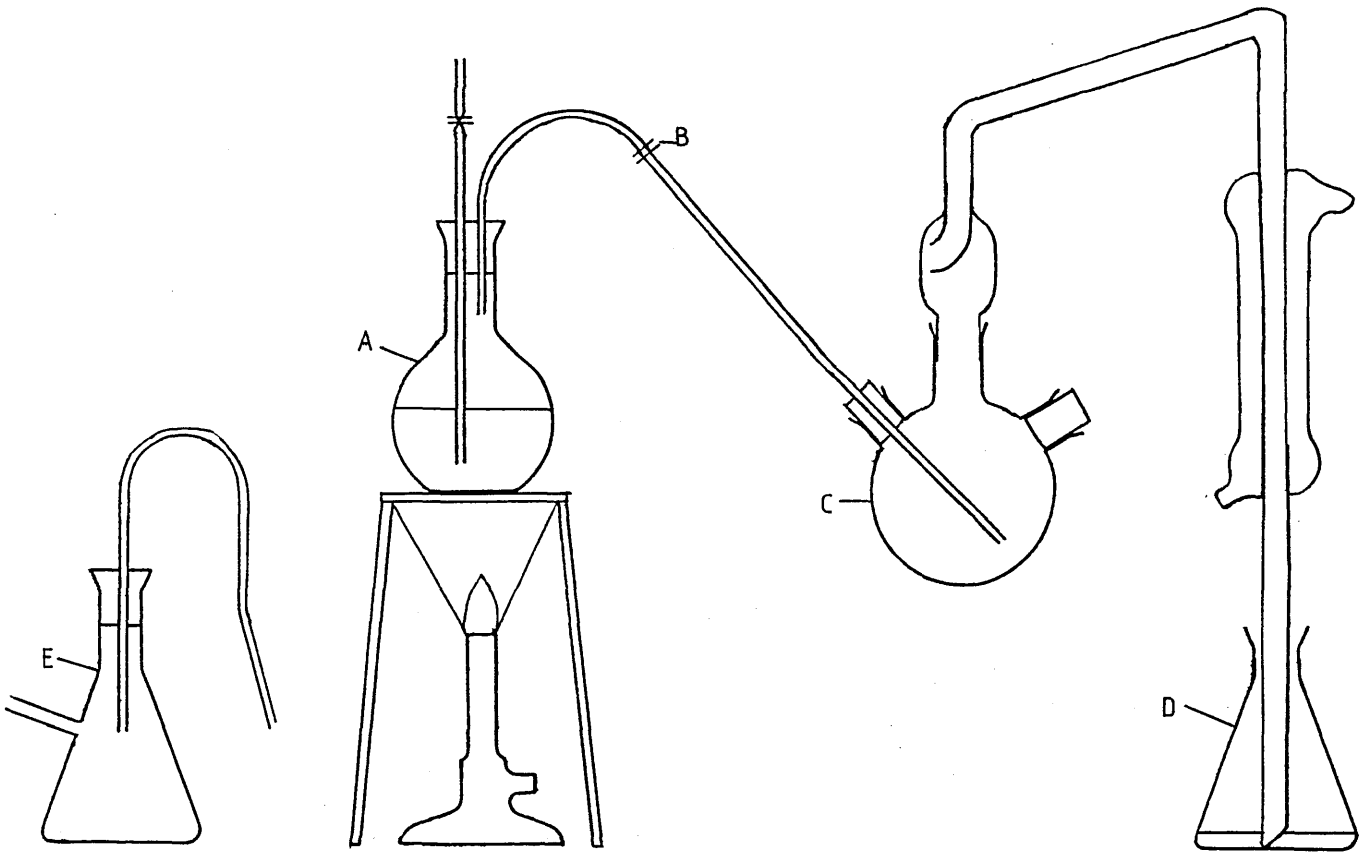
Where C = the concentration of the element (ug cm^{-3}) in flask A.

This value was corrected, where necessary, as described in section 2.5.3 to give the %w/w of copper, chromium and arsenic based on the initial dry weight of the block.

2.5.5 Determination of the nitrogen as ammonia contents.

The apparatus employed in the determination of the ammonium nitrogen content of the wood is shown in figure 2.6. The method used was that developed by Briscoe (1987).

Blocks were reduced to small, short sticks using a Stanley knife and a randomly selected portion (approximately 0.1g) was collected and accurately weighed. Samples of buried wood blocks had been dried



A = 2 litre flask.

B = Clip.

C = 3-necked flask.

D = 100cm^3 flask + 5cm^3 2% boric acid & 10 drops Kjeldahl indicator.

E = Buchner flask and water pump.

Figure 2.6 Apparatus used in nitrogen as ammonia determinations.

to constant weight at 102±2°C prior to weighing. The moisture content of the unburied wood blocks was determined on a separate sample of wood and used to correct the weight of the sample taken for ammonium nitrogen determination to a dry weight.

Water was brought to a boil in a flat bottomed 2 litre flask (A) and used to steam the apparatus for at least 10 minutes prior to its use. The apparatus was rinsed well with distilled water prior to any determination.

The bunsen burner was removed from beneath the boiling water and the clip (B) closed, preventing steam from going through the system. The stopper was removed from the three-necked flask (C) and the wood sample placed in the flask. $10cm^3$ of 40%w/v sodium hydroxide was added, the wood chips were washed to the bottom of the flask using distilled water, and the stopper replaced. The clip was then released and the heat re-applied to the flask. This procedure was carried out as rapidly as possible.

A $100cm^3$ conical flask containing $5cm^3$ of 2%w/v boric acid solution and 10 drops of Kjeldahl indicator (D) was placed beneath the condenser and $20cm^3$ of distillate was collected and the solution titrated against standard 0.01M hydrochloric acid solution.

After each distillation, flask C was emptied using the vacuum produced by a water pump connected to a Buchner flask (E). The flask was then rinsed with distilled water and the rinsings removed in the same manner.

The %w/w ammonium nitrogen content of the wood sample was calculated using the equation for the calculation of the %w/w nitrogen content of wood (section 2.5.3).

2.5.6 Measurement of the dehydrogenase level in wood.

The assay of dehydrogenase activity in the outer wood surface and inner wood of buried wood blocks was similar to that previously described for soil (section 2.4.2). $2cm^3$ of 0.75%w/v TTC aqueous solution was added to the accurately weighed wood sample (section 2,3,3), the sealed test tube shaken, to ensure even distribution of the solution, and then incubated, along with the soil samples, at 30°C.

The solvent extraction regimes, previously described in section 2.4.2, were employed in the course of the appropriate experimental programme to remove TTF from the wood. The concentration of TTF was determined (section 2.4.2) and, once the weights of the assayed wood samples had been corrected to a dry weight using the moisture content of the dried portion of inner wood, the level of dehydrogenase activity was calculated using the equation,

$$\text{umol TTF } g^{-1} \text{ } min^{-1} = \frac{(\text{umol } cm^{-3}) * 10}{[\text{weight of wood (dry)}] * (24*60)}$$

2.5.7 Experimental check on the dehydrogenase method.

In the course of experimental programmes 2 and 3, it was observed that decaying, preservative treated blocks had lower dehydrogenase levels for their rate of weight loss than had similar untreated blocks. To ascertain whether this was due to genuine lower levels of microbial activity or to interference from the preservative, the experiment described below was carried out using the following samples,

a. soil of the type used in all soil burial studies,

b. untreated pine, spruce and lime,

c. 0.25%w/v CCA-treated pine and spruce, 3%w/v CCA-treated lime, 5%w/v CCA-treated spruce,

d. 0.07 and 1.41%w/v ACA-treated spruce, 0.14%w/v ACA-treated lime.

0.1g samples of finely divided, dried wood samples, or 1.5g samples of dried soil were weighed and placed in a screw-capped test tube (12*1.5cm), which already contained 15mg calcium carbonate. $2cm^3$ of water was added and the sealed tubes were then autoclaved (121°C, 15psi), ensuring penetration of water into the test sample. Once the solutions were cool, $2cm^3$ of a known dilution of TTF in methanol was added to each tube. Control tubes of 15mg calcium carbonate, $2cm^3$ water and $2cm^3$ of TTF in methanol also set up. 5 replicates of all test samples and controls were prepared. On mixing the TTF solution with water the formation of a red precipitate was immediately observed. The sealed tubes were incubated in the dark at 30°C for 24 hours.

Following incubation the TTF was extracted from the experimental and control tubes using either solvent extraction regime I or II (section 2.4.2) and the absorbance measured as previously described (section 2.4.2). It should be noted that all wood samples had been buried in soil, usually for a period of 6 weeks, so that unfixed preservative could not colour the solutions. The absorbance values obtained for the test samples were compared with those of the controls.

2.6 Statistical analyses employed.

A number of statistical methods have been used throughout the current work and brief details of these are presented here.

F and T-tests.

To determine whether differences between two groups of data could have arisen by chance the results were analysed statistically using a T-test. Before the T-test was carried out, an F-test was employed to ascertain whether the standard deviations of the two groups differed significantly. If the standard deviations were significantly different the T-test could not be carried out, as this test assumes that there are comparable variabilities within the two groups. F and T-tests were carried out using a statistical package on the Sinclair ZX Spectrum.

One and two-way analyses of variance.

The one-way analysis of variance can be considered to be a test of whether two or more sample means could have been obtained from populations with the same parametric means with respect to a given variable (Sokal and Rohlf, 1973). In the current work the one-way analysis of variance was always used to compare more than two sample means, since the comparison of two averages could be carried out using the T-test.

The assumption of the two-way analysis of variance is that two contributions, e.g., time and leaching, add their effects, e.g., to increases in wood nitrogen content, without influencing each other (Sokal and Rohlf, 1973 *op cit*). The level of significance of a third factor, known as the interaction mean square, is also calculated during this method. Interaction occurs when the effect of the two contributions applied together cannot be predicted from the average responses of the separate contributions, e.g., if two different wood treatments gave separate, non-parallel curves for a measured parameter, then a highly significant interaction mean square would be expected, however, if the curves for the two wood treatments were

parallel, no significant interaction would be predicted. Where a highly significant interaction mean square is obtained, the two populations are clearly reacting to the two contributory factors in different ways, therefore, an overall statement for the effect of each factor would have little meaning. For this reason many statisticians would not even test the effect of the two contributory factors once they found the interaction mean square to be significant (Sokal and Rohlf, 1973 *op cit*), though in such cases these calculations were carried out in the current work.

One and two-way analyses of variance were carried out using the Minitab statistical package (Copyright - Minitab, Inc. 1985) on the Vax-system.

The Quade test.

Where two groups of data had very different standard deviations use of the two-way analysis of variance was precluded. In such cases the Quade test (Conover, 1980), a non-parametric test based on ranking, was employed. Analyses were carried out using the formulae presented in Conover (1980 *op cit*).

Table 2.4 Summary of the experimental methods used during each experimental programme.

Experimental procedure	Methods section	Experimental programme 1(1)	1(2)	2	3
Wood blocks prepared.	2.2	X	X	X	X
Wood blocks treated with,					
i. CCA	2.2.1	X	X	X	
ii. ACA	& 2.2.2				X
Leach studies carried out on unburied blocks.	2.2.3	X			X
Analysis of leach liquors,					
i. for preservative metals.	2.2.4	X			X
ii. for nitrogen levels.	2.2.4				X
Leached blocks included in the soil burial study.	2.2.3	X	X		
Soil adjacent to buried blocks analysed for,					
i. levels of preservative metals.	2.4.1	X			X
ii. levels of dehydrogenase activity.	2.4.2			X	X
Analysis of wood blocks for levels of,					
a. moisture.	2.5.1	X	X	X	X
b. weight loss.	2.5.2	X	X	X	X
c. nitrogen.	2.5.3	X	X	X	X
d. preservative metals.	2.5.4	X			X
e. nitrogen as ammonia.	2.5.5				X
f. dehydrogenase	2.5.6			X	X

Key. X procedure carried out in the course of the experimental programme.

CHAPTER 3

RESULTS

RESULTS

3.1 Experimental programme 1.

Losses of preservative metals from CCA-treated wood blocks during leaching and soil burial, and the effect of pre-burial leaching on subsequent moisture uptake by wood blocks during soil burial.

3.1.1 Cold water leach studies.

3.1.1.1 Introduction.

The results of two cold water leach studies carried out on untreated and CCA-treated wood blocks are presented in this section. In the first leaching experiment, blocks which had been treated at the same time as those used in the first soil burial study of this experimental programme were leached, as were untreated blocks. The copper and chromium contents of the daily leach liquors were determined (figures 3.1.1.1-3.1.1.6). In the second leaching experiment, blocks which had been treated at a later date were leached allowing the arsenic content of the leach liquors to be determined (figures 3.1.1.1-3.1.1.6).

Two methods are used to express the extent of preservative metal losses during leaching: (i) the comparison method and (ii) the addition method.

(i) The comparison method.

$$\text{Loss of preservative metals (\%)} = \frac{(A-B)}{A} * 100$$

where, A is the preservative metal present in unleached blocks (ug) [total of 6 replicate blocks], and

B is the preservative metal remaining in leached blocks (ug) [total of 6 replicate blocks].

Statistical analyses (F and T-tests, see section 2.6) of the data were carried out to determine if any measured losses were significant. The results of these calculations and the wood preservative metal content data are presented in section 3.1.1.2.

(ii) The addition method. (based on Norton, 1979)

$$\text{Loss of preservative metals (\%)} = \frac{C}{(B+C)} * 100$$

where, B is the preservative metal remaining in leached blocks (ug) [total of 6 replicate blocks], and
C is the preservative metal present in duplicate sets of leach liquors (ug) [total of 5 days].

The results of this calculation are presented in section 3.1.1.4.

The copper, chromium and arsenic contents of the daily leach liquors used to leach the untreated and CCA-treated wood blocks are presented in section 3.1.1.3. The nitrogen contents of untreated and CCA-treated blocks leached during the first leaching experiment and their unleached counterparts were determined and the results are given in section 3.1.1.5.

3.1.1.2 Wood preservative metal contents and leach losses (%) of preservative metals as calculated by the comparison method.

The copper, chromium and arsenic contents of unleached and leached, CCA-treated blocks calculated from liquid uptake data and by chemical analysis are presented in tables 3.1.1.1-3.1.1.3. The copper and chromium contents determined by chemical analysis of unleached and leached, untreated blocks are presented in table 3.1.1.4. The

arsenic contents of the untreated blocks were not determined.

CCA leaching experiment 1.

Leach losses of the preservative metals calculated by the comparison method and the results of statistical analyses are presented in table 3.1.1.5. The average metal contents of the leached blocks, as determined by chemical analysis, were frequently greater than those of the corresponding unleached blocks (table 3.1.1.5). However, although the average metal content of these leached blocks based on their preservative liquid uptake was also greater, the reverse was not always true (tables 3.1.1.1-3.1.1.3).

By the comparison method all CCA-treated spruce blocks suffered losses of preservative metals during leaching (table 3.1.1.5). Leach losses of arsenic from the 3 and 5%w/v CCA-treated spruce blocks were particularly large, though only in the latter case was the decrease statistically significant. Where the preservative metal contents of other leached, CCA-treated groups of wood blocks were less than those of their unleached counterparts, the difference was never greater than 10%, and was not statistically significant (table 3.1.1.5).

CCA leaching experiment 2.

Calculated preservative retentions of blocks prepared for leaching experiment 2 were much greater than those of the comparable blocks which constituted leaching experiment 1 (tables 3.1.1.1-3.1.1.4), this difference being in the order of between +10 and 20%. This difference was also shown by chemical analysis (tables 3.1.1.1-3.1.1.3). The only known difference in the preservative treatment between the two sets of blocks was that the vacuum desiccator used in the impregnation procedure had been changed. This may indicate an improved preservative penetration due to the new

vacuum desiccator, thus increasing preservative retentions. Thus wood preservative metal contents from the two leaching experiments cannot be directly compared.

3.1.1.3 Preservative metal contents of daily leach liquors used to leach untreated and CCA-treated wood blocks.

In both leaching experiments the preservative metal contents of duplicate daily leach liquors were determined. The median value for each pair of leach liquors are shown in figures 3.1.1.1-3.1.1.6.

CCA leaching experiment 1.

Copper contents of control leach liquors from the untreated blocks varied between 16 and 52ug. Chromium levels of these liquors were between 0 and 12ug.

The greatest loss of copper from CCA-treated wood blocks occurred during the first day of leaching (figures 3.1.1.1-3.1.1.6), accounting for more than a third of the copper lost during five days of leaching. After two to three days small, relatively consistent amounts of copper were leached from each set of replicate blocks (figures 3.1.1.1-3.1.1.6). The levels of copper leached from the CCA-treated softwood blocks were similar for both wood species and preservative treatments (figures 3.1.1.1-3.1.1.4). More copper was leached daily from the CCA-treated lime blocks than from the softwood blocks, though very little difference was observed between the two different preservative concentrations.

The amount of chromium leached daily from the CCA-treated wood blocks was always lower than the corresponding amount of copper content (figures 3.1.1.1-3.1.1.6). The greatest daily amount of chromium leached occurred during the first day, with the exception of 3%w/v CCA-treated spruce, where most was leached during the fifth day

(figures 3.1.1.1- 3.1.1.6). Although after the first two days of leaching, only minimal quantities of chromium were detected in the leach liquors, these levels were still greater than the control levels.

As most of the preservative metal losses occurred during the first two days of leaching, with only small amounts being leached afterwards, the leaching procedure was terminated after five days. CCA leaching experiment 2.

The copper and arsenic concentrations in the distilled water used in this experiment were found to be $6.85*10^{-3} \pm 1.65*10^{-3}$ $ugcm^{-3}$ and $1.18*10^{-3} \pm 2.03*10^{-3}$ $ugcm^{-3}$ respectively, which is equivalent to approximately 4ug of copper and lug of arsenic per daily leach liquor. No chromium was detected.

As in experiment 1, the greatest levels of copper and chromium losses occurred during the first day of leaching (figures 3.1.1.1-3.1.1.6). Leach losses of copper from CCA-treated lime and softwood blocks were greater than in the first experiment, however the difference between the two experiments was greatest for the softwoods. Leach losses of chromium from CCA-treated pine and lime blocks were also greater in this experiment, particularly for 5%w/v CCA-treated pine. However, there was little difference in the chromium levels in the spruce leach liquors in the two experiments.

The greatest arsenic contents were measured in leach liquors which had been in contact with CCA-treated lime blocks (figures 3.1.1.1-3.1.1.6). In general, the levels of arsenic measured in each set of leach liquors were lower than those of copper and slightly greater than levels of chromium.

3.1.1.4 Leach losses of preservative metals (%) from CCA-treated wood blocks as calculated by the addition method.

The percentage losses of copper, chromium and arsenic from the CCA-treated blocks to the leaching water calculated by the addition method (see section 3.1.1.1) are presented in table 3.1.1.6.

CCA leaching experiment 1.

Percentage losses of copper from 3 and 5%w/v CCA-treated lime blocks were very similar (table 3.1.1.6), and constituted the largest losses of any element measured during this experiment. CCA-treated softwoods had substantially lower percentage losses of copper than the lime blocks. Slightly greater losses generally occurred from the 3%w/v CCA-treated softwood blocks than from the 5%w/v CCA-treated softwood blocks (table 3.1.1.6). Leach losses of chromium from all CCA-treated blocks were lower than those of copper.

CCA leaching experiment 2.

Leach losses of copper and chromium from CCA-treated lime blocks were similar to those obtained in the first leaching study (table 3.1.1.6). However, losses of copper from CCA-treated softwood blocks were up to 5 times greater (table 3.1.1.6). The loss of copper from the 5%w/v CCA-treated pine blocks was the greatest observed from any type of CCA-treated block in either study. The CCA-treated spruce blocks had slightly lower leach losses of chromium in this experiment, while chromium losses from the CCA-treated pine blocks were somewhat greater. Of the arsenic originally present in the CCA-treated blocks, less than 5% was leached, irrespective of wood species and preservative treatments (table 3.1.1.6). The greatest losses of arsenic were from the CCA-treated lime blocks (table 3.1.1.6). CCA-treated softwood blocks lost less than 2% of their

original arsenic content to the leach liquors (table 3.1.1.6).

Losses of metals from CCA-treated blocks calculated by the addition method show the leaching order to be,

copper > arsenic > chromium.

3.1.1.5 Wood block nitrogen contents.

The nitrogen contents of the untreated and CCA-treated wood blocks of CCA leaching experiment 1 are presented in table 3.1.1.7. The nitrogen contents of all replicate groups of softwood blocks were similar (table 3.1.1.7). Lime nitrogen contents were always greater than those of the softwoods. Of the nine groups of unleached and leached blocks, there was a decrease in the average nitrogen content in five cases (table 3.1.1.7), though generally the differences between the averages were small. On three occasions the leached blocks had a slightly greater average nitrogen content than the corresponding unleached group of blocks (table 3.1.1.7).

F and T-tests were used to statistically compare the nitrogen data of the corresponding unleached and leached groups of blocks (section 2.6). It was found that a T-test was justified for every comparison. In eight of the nine T-tests no statistically significant difference was found between the nitrogen contents of the unleached and leached groups of blocks. The exception was the 5%w/v CCA-treated lime blocks, where a slightly significant increase ($p < 5\%$) in the wood nitrogen contents on leaching was indicated.

3.1.1.6 Figures 3.1.1.1-3.1.1.6

Figure 3.1.1.1 Preservative metal contents of daily leach liquors from 3%w/v CCA-treated pine blocks during leaching experiments 1 and 2.

Figure 3.1.1.2 Preservative metal contents of daily leach liquors from 5%w/v CCA-treated pine blocks during leaching experiments 1 and 2.

Figure 3.1.1.3 Preservative metal contents of daily leach liquors from 3% w/v CCA-treated spruce blocks during leaching experiments 1 and 2.

Figure 3.1.1.4 Preservative metal contents of daily leach liquors from 5%w/v CCA-treated spruce blocks during leaching experiments 1 and 2.

Figure 3.1.1.5 Preservative metal contents of daily leach liquors from 3%w/v CCA-treated lime blocks during leaching experiments 1 and 2.

Figure 3.1.1.6 Preservative metal contents of daily leach liquors from 5%w/v CCA-treated lime blocks during leaching experiments 1 and 2.

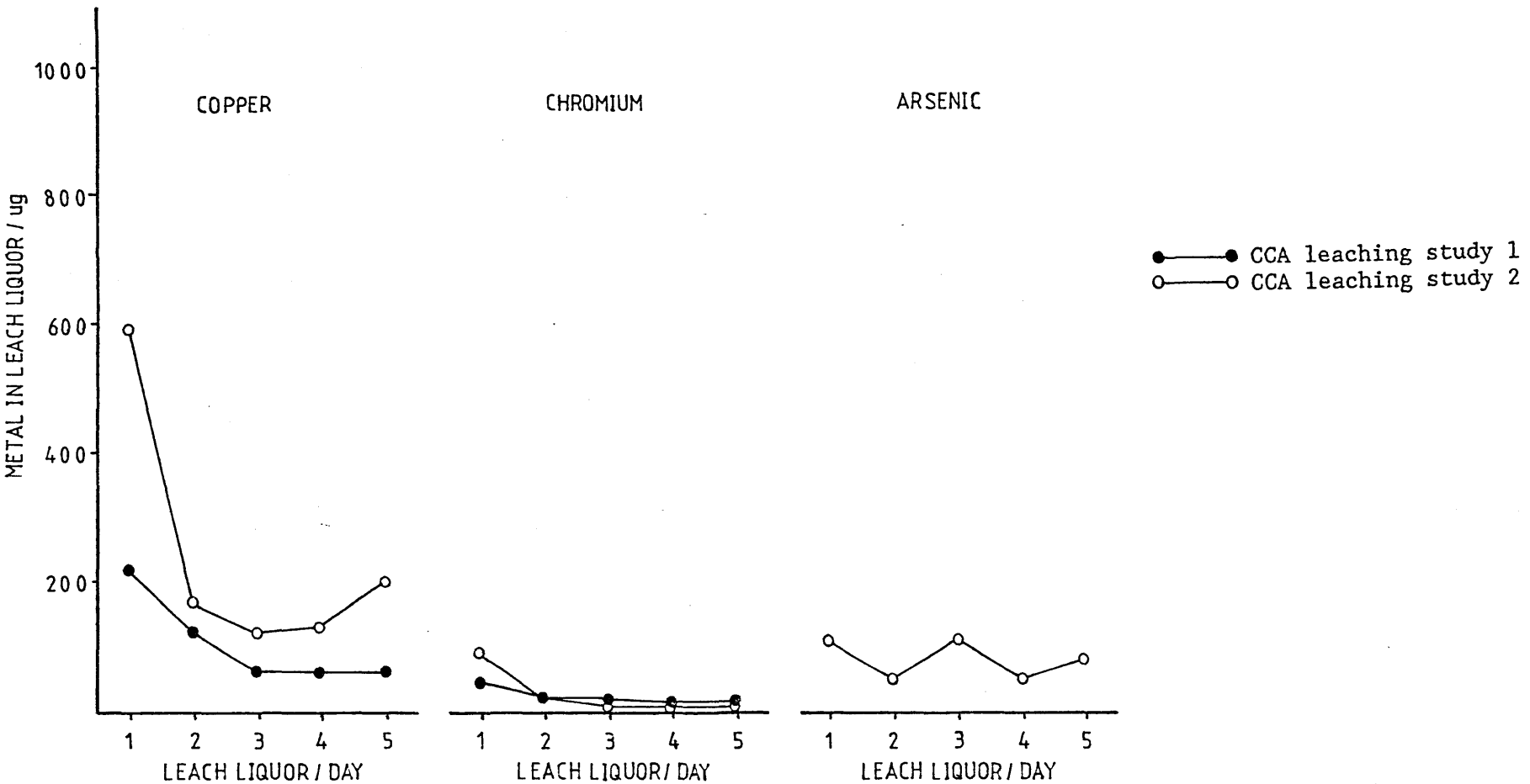


Figure 3.1.1.1 Preservative metal contents of daily leach liquors from 3%w/v CCA-treated pine blocks during leaching experiments 1 and 2. Median levels in duplicate leach liquors are shown.

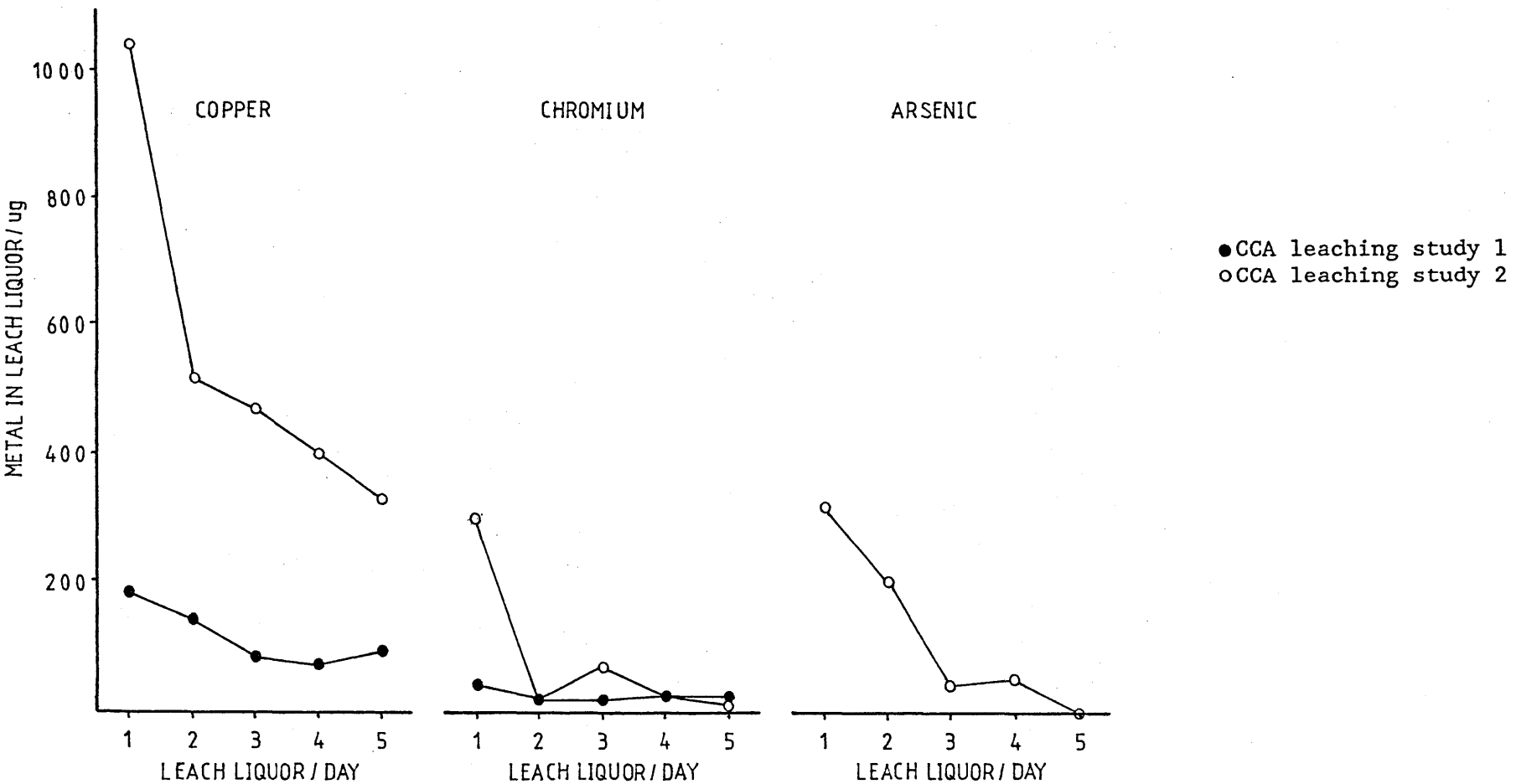


Figure 3.1.1.2 Preservative metal contents of daily leach liquors from 5%w/v CCA-treated pine blocks during leaching experiments 1 and 2. Median levels in duplicate leach liquors are shown.

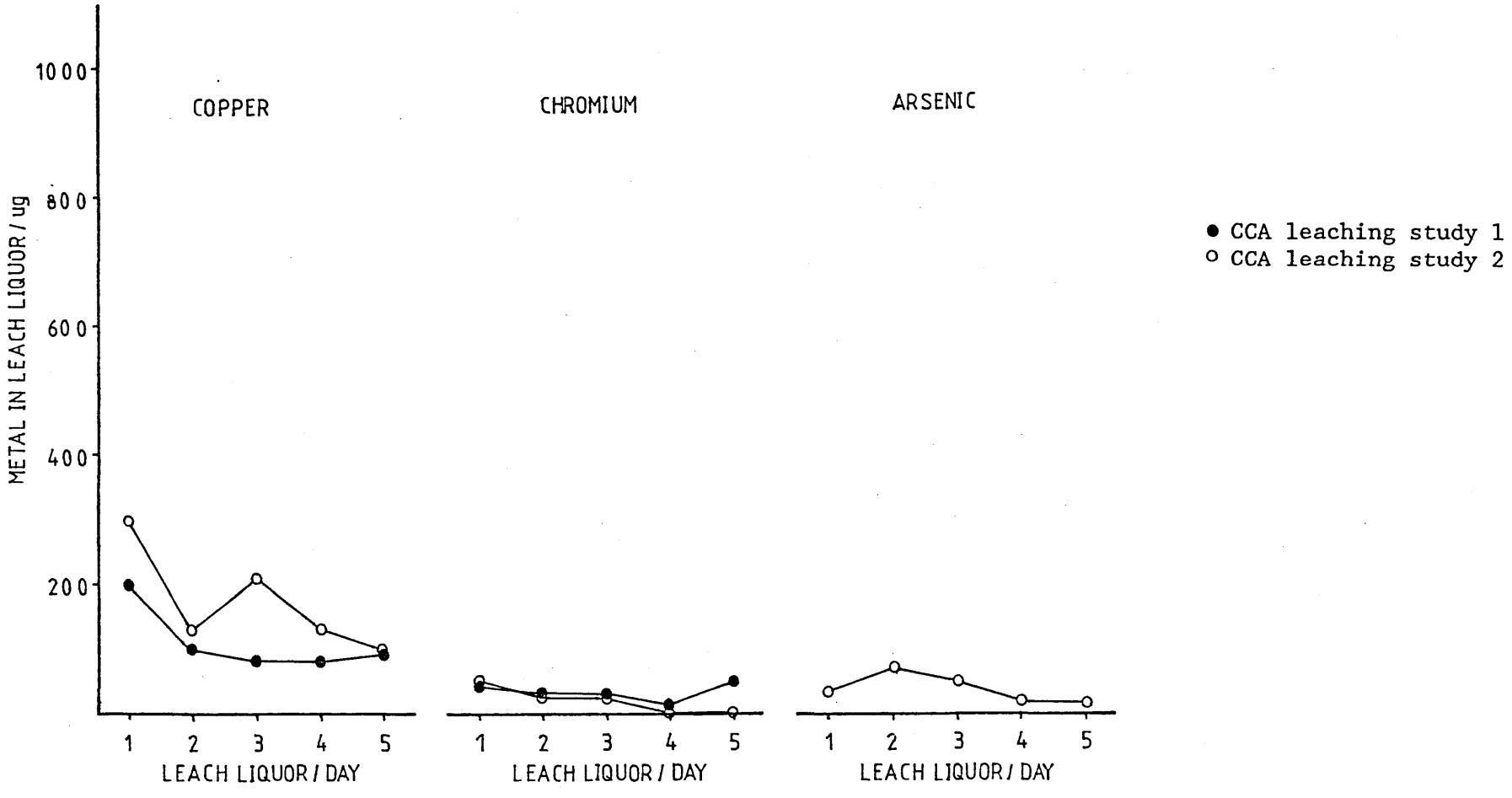


Figure 3.1.1.3 Preservative metal contents of daily leach liquors from 3%w/v CCA-treated spruce blocks during leaching experiments 1 and 2. Median levels in duplicate leach liquors are shown.

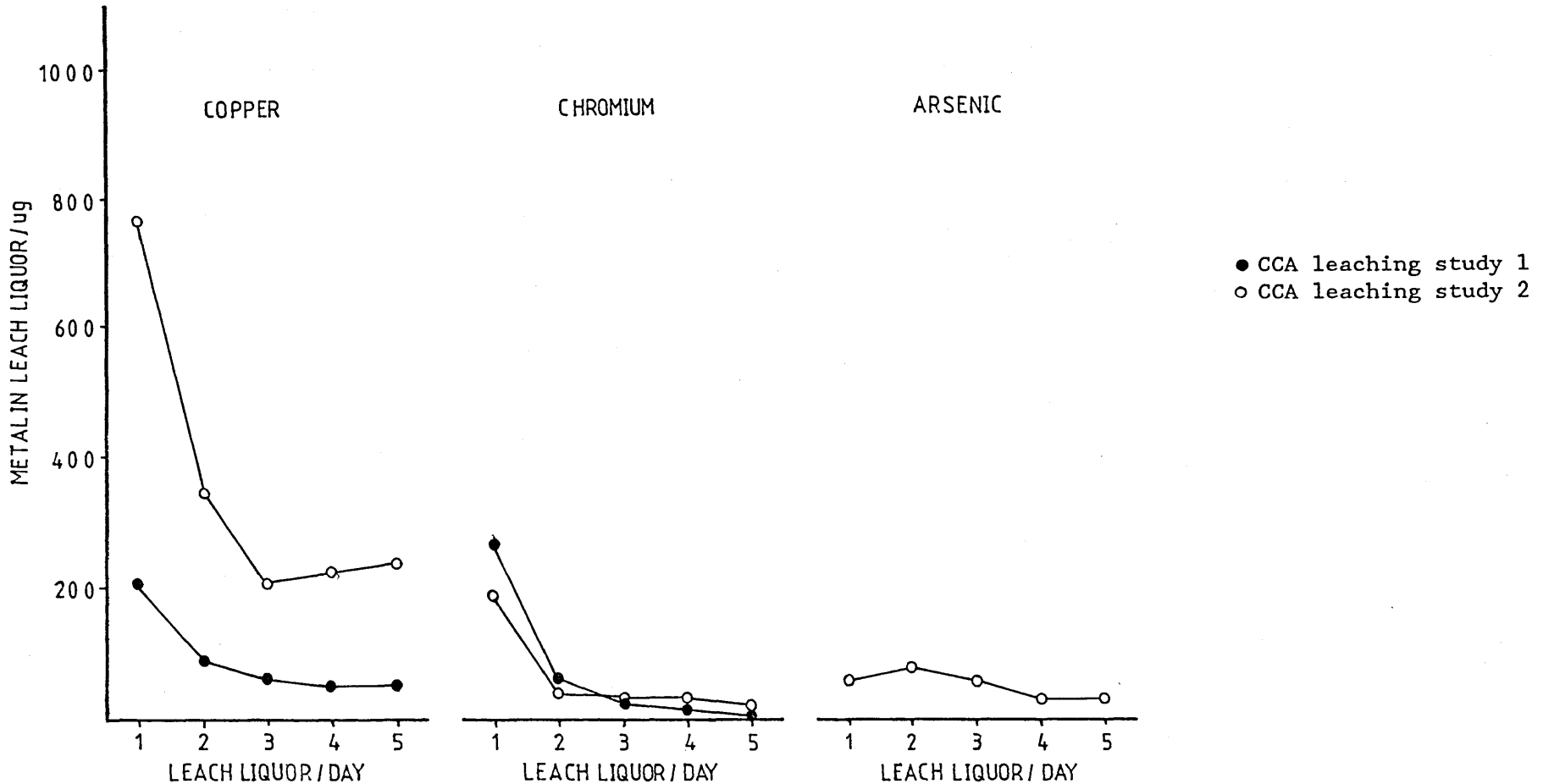


Figure 3.1.1.4 Preservative metal contents of daily leach liquors from 5%w/v CCA-treated spruce blocks during leaching experiments 1 and 2. Median levels in duplicate leach liquors are shown.

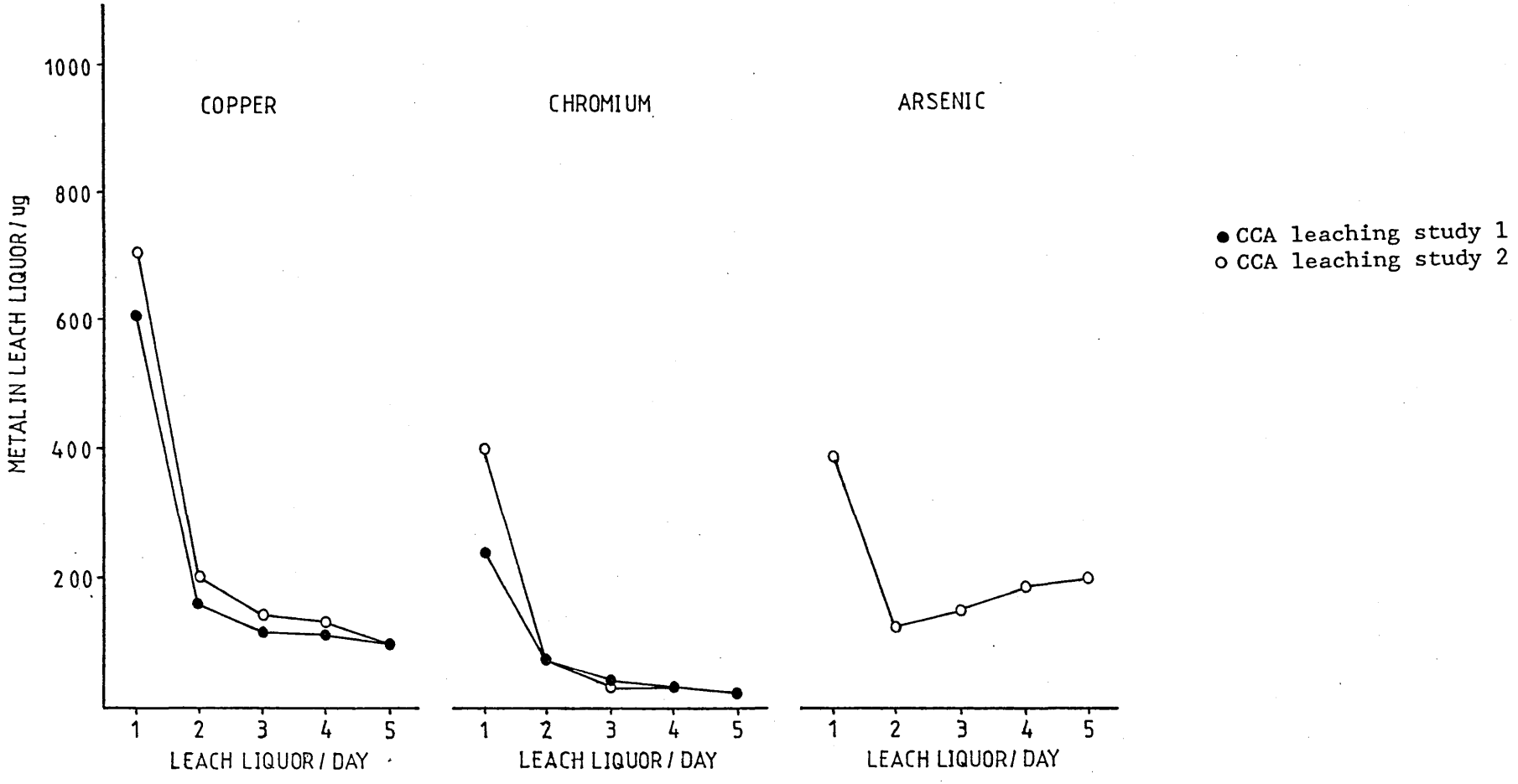


Figure 3.1.1.5 Preservative metal contents of daily leach liquors from 3%w/v CCA-treated lime blocks during leaching experiments 1 and 2. Median levels in duplicate leach liquors are shown.

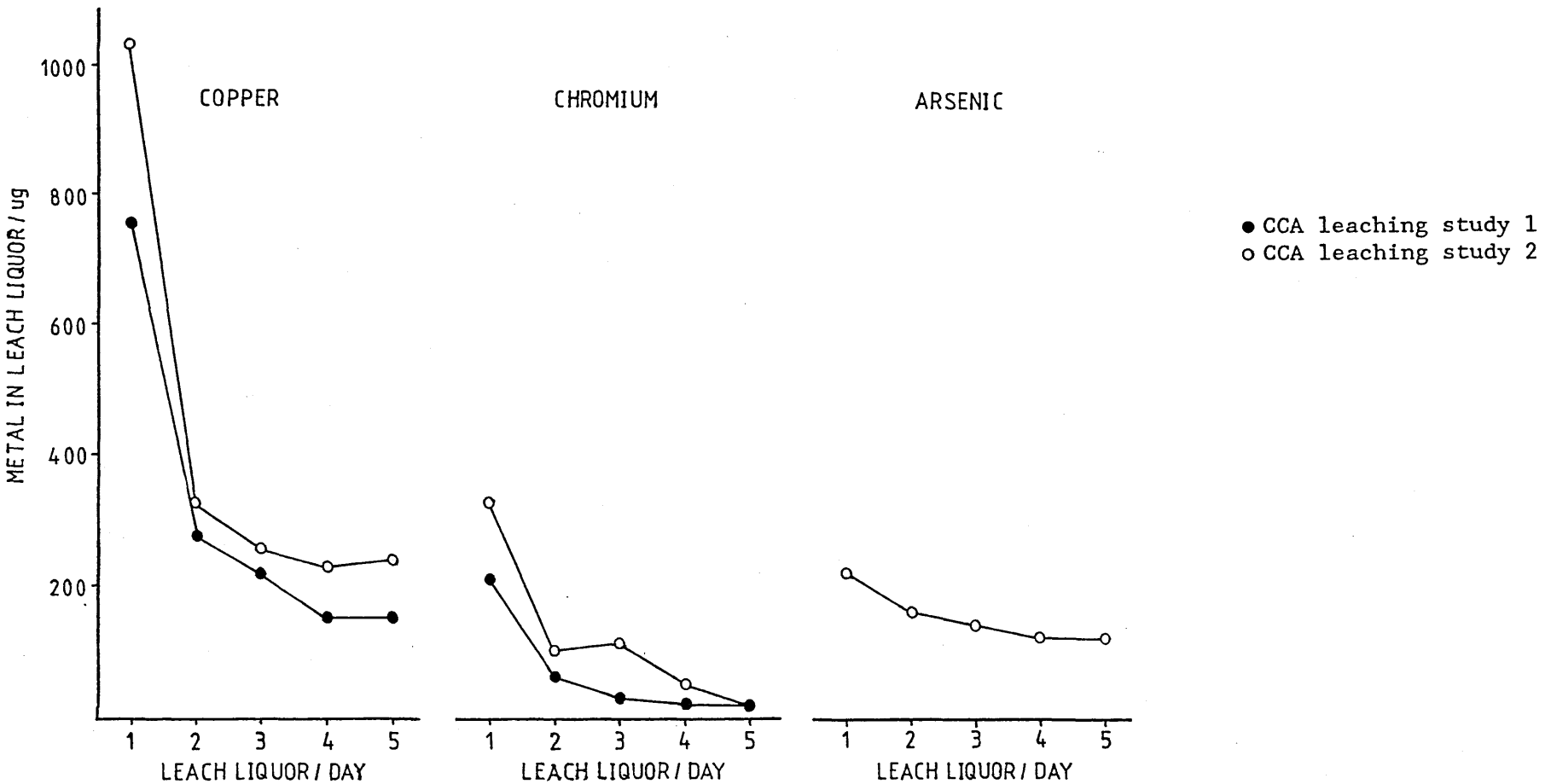


Figure 3.1.1.6 Preservative metal contents of daily leach liquors from 5%w/v CCA-treated lime blocks during leaching experiments 1 and 2. Median levels in duplicate leach liquors are shown.

3.1.1.7 Tables 3.1.1.1-3.1.1.7.

Table 3.1.1.1 Copper contents of CCA-treated wood blocks (leaching experiments 1 and 2).

Table 3.1.1.2 Chromium contents of CCA-treated wood blocks (leaching experiments 1 and 2).

Table 3.1.1.3 Arsenic contents of CCA-treated wood blocks (leaching experiments 1 and 2).

Table 3.1.1.4 Copper and chromium contents of untreated wood blocks (leaching experiment 1).

Table 3.1.1.5 Preservative metal leach losses from CCA-treated wood blocks calculated by the comparison method (leaching experiment 1 only). Also results of T-tests on corresponding leached and unleached blocks.

Table 3.1.1.6 Preservative metal leach losses from CCA-treated wood blocks calculated by the addition method.

Table 3.1.1.7 Nitrogen contents of wood blocks from CCA leaching experiment 1.

Table 3.1.1.1 Copper contents of CCA-treated wood blocks (leaching experiments 1 and 2). Data is based on liquid uptake values and analytical data. Mean results ± standard deviations are presented (mean is based on a minimum of 5 replicates).

Wood species	Leaching	Expt. no.		Copper content (%w/w)	
				3%w/v CCA	5%w/v CCA
Pine	Not leached	1	liquid uptake	0.347±0.027	0.580±0.018
			analysis	0.350±0.032	0.552±0.048
	Leached	1	liquid uptake	0.359±0.014	0.605±0.015
			analysis	0.401±0.052	0.630±0.046
	Leached	2	liquid uptake	0.413±0.041	0.652±0.012
			analysis	0.433+0.043	0.634+0.037
Spruce	Not leached	1	liquid uptake	0.558±0.068	0.932±0.077
			analysis	0.596±0.053	0.905±0.081
	Leached	1	liquid uptake	0.534±0.018	0.818±0.145
			analysis	0.546±0.032	0.819±0.129
	Leached	2	liquid uptake	0.627±0.069	1.018±0.067
			analysis	0.614+0.079	1.054+0.124
Lime	Not leached	1	liquid uptake	0.299±0.013	0.469±0.042
			analysis	0.294±0.013	0.439±0.035
	Leached	1	liquid uptake	0.306±0.021	0.476±0.023
			analysis	0.309±0.031	0.402±0.030
	Leached	2	liquid uptake	0.345±0.013	0.558±0.022
			analysis	0.364+0.011	0.582+0.039

Table 3.1.1.2 Chromium contents of CCA-treated wood blocks (leaching experiments 1 and 2). Data is based on liquid uptake values and analytical data. Mean results ± standard deviations are presented (mean is based on a minimum of 5 replicates).

Wood species	Leaching	Expt. no.		Chromium content (%w/w)	
				3%w/v CCA	5%w/v CCA
Pine	Not leached	1	liquid uptake	0.656±0.050	1.098±0.035
			analysis	0.682±0.054	1.084±0.057
	Leached	1	liquid uptake	0.680±0.026	1.146±0.028
			analysis	0.786±0.110	1.157±0.047
	Leached	2	liquid uptake	0.771±0.067	1.234±0.022
			analysis	0.797+0.059	1.214+0.068
Spruce	Not leached	1	liquid uptake	1.060±0.132	1.766±0.146
			analysis	1.108±0.112	1.699±0.177
	Leached	1	liquid uptake	1.010±0.034	1.551±0.274
			analysis	1.034±0.076	1.551±0.274
	Leached	2	liquid uptake	1.187±0.130	1.923±0.121
			analysis	1.178+0.141	1.992+0.197
Lime	Not leached	1	liquid uptake	0.566±0.025	0.888±0.079
			analysis	0.575±0.038	0.961±0.061
	Leached	1	liquid uptake	0.580±0.039	0.911±0.036
			analysis	0.588±0.034	0.937±0.072
	Leached	2	liquid uptake	0.653±0.024	1.058±0.042
			analysis	0.796+0.076	1.277+0.201

Table 3.1.1.3 Arsenic contents of CCA-treated wood blocks (leaching experiments 1 and 2). Data is based on liquid uptake values and analytical data. Mean results ± standard deviations are presented (mean is based on a minimum of 5 replicates).

Wood species	Leaching	Expt. no.		Arsenic content (%w/w)	
				3%w/v CCA	5%w/v CCA
Pine	Not leached	1	liquid uptake	0.452±0.035	0.761±0.024
			analysis	0.444±0.057	0.921±0.141
	Leached	1	liquid uptake	0.468±0.018	0.794±0.019
			analysis	0.428±0.042	1.032±0.043
	Leached	2	liquid uptake	0.531±0.046	0.855±0.015
			analysis	0.529+0.050	0.920+0.053
Spruce	Not leached	1	liquid uptake	0.727±0.089	1.223±0.101
			analysis	0.881±0.078	1.484±0.254
	Leached	1	liquid uptake	0.696±0.023	1.074±0.190
			analysis	0.757±0.129	1.162±0.212
	Leached	2	liquid uptake	0.818±0.089	1.336±0.087
			analysis	0.808+0.135	1.404+0.237
Lime	Not leached	1	liquid uptake	0.390±0.017	0.615±0.055
			analysis	0.460±0.087	0.746±0.073
	Leached	1	liquid uptake	0.400±0.027	0.631±0.025
			analysis	0.554±0.158	0.713±0.096
	Leached	2	liquid uptake	0.451±0.015	0.733±0.029
			analysis	0.482+0.069	0.786+0.102

Table 3.1.1.4 Copper and chromium contents of untreated wood blocks (leaching experiment 1). Mean results ± standard deviations are presented (mean is based on a minimum of 5 replicates).

Wood species	Leaching	Copper content (%w/w)	Chromium content (%w/w)
Pine	Not leached	0.009±0.002	0.003±0.001
	Leached	0.015±0.003	0.013±0.019
Spruce	Not leached	0.011±0.004	0.003±0.002
	Leached	0.009±0.002	0.006±0.004
Lime	Not leached	0.009±0.002	0.001±0.001
	Leached	0.014±0.002	0.004±0.002

Table 3.1.1.5 Preservative metal leach losses from CCA-treated wood blocks calculated by the comparison method (leaching experiment 1 only). Results of a T-test carried out on corresponding unleached and leached groups of blocks are also presented.

Wood species	Metal	3%w/v CCA		5%w/v CCA	
		Loss	T-test	Loss	T-test
Pine	Copper	Inc	NS	Inc	*
	Chromium	Inc	NS	Inc	*
	Arsenic	3.6	NS	Inc	↯
Spruce	Copper	8.4	NS	9.5	NS
	Chromium	6.7	NS	8.7	NS
	Arsenic	14.1	NS	21.7	*
Lime	Copper	Inc	↯	8.4	NS
	Chromium	Inc	NS	2.5	NS
	Arsenic	Inc	NS	4.4	NS

Key Inc Mean metal content of leached blocks is greater than that of unleached blocks.

↯ F-test indicates that the T-test cannot be carried out.

NS No significant difference.

* Significant difference: probability of the difference arising by chance is < 5%.

Table 3.1.1.6 Preservative metal leach losses from CCA-treated wood blocks calculated by the addition method.

Wood species	Expt. no.	Preservative concentration	Leach losses (%)		
			Copper	Chromium	Arsenic
Pine	1	3%w/v	2.83	0.26	M/N
		5%w/v	1.90	0.20	M/N
	2	3%w/v	5.83	0.38	1.90
		5%w/v	9.47	1.59	1.30
Spruce	1	3%w/v	3.48	0.53	M/N
		5%w/v	2.07	0.88	M/N
	2	3%w/v	5.20	0.30	0.89
		5%w/v	5.20	0.57	0.72
Lime	1	3%w/v	7.20	1.48	M/N
		5%w/v	7.64	0.80	M/N
	2	3%w/v	7.19	1.51	4.61
		5%w/v	7.27	1.05	2.09

Key M/N Appropriate measurements not carried out.

Table 3.1.1.7 Nitrogen contents of wood blocks from CCA leaching experiment 1. Mean results ± standard deviations are presented (mean is based on a minimum of 5 replicates).

Wood species	Leaching	Nitrogen content (%w/w)		
		Untreated	3%w/v CCA	5%w/v CCA
Pine	Not leached	0.049±0.005	0.049±0.005	0.045±0.002
	Leached	0.047±0.006	0.048±0.006	0.049±0.005
Spruce	Not leached	0.046±0.005	0.047±0.008	0.054±0.004
	Leached	0.047±0.003	0.047±0.006	0.048±0.005
Lime	Not leached	0.098±0.029	0.106±0.019	0.093±0.017
	Leached	0.090±0.039	0.091±0.019	0.119±0.019

3.1.2 Soil burial experiment 1.

Assessment of preservative metal losses CCA-treated wood blocks and their accumulation in adjacent soil during soil burial.

The statistical significance of change in parameters, such as wood nitrogen and preservative metal contents, soil preservative metal contents, etc, with time were determined using one-way analysis of variance (see section 2.6). Additional statistical analyses using a two-way analysis of variance were carried out on some data to enable further interpretation of these results. Results of these statistical analyses are presented in the appropriate sections.

3.1.2.1 Moisture contents of buried wood blocks.

Average wood moisture contents and standard deviations, are presented in table 3.1.2.1. Average results for all pine blocks are shown in figure 3.1.2.1. For the sake of clarity, standard deviations are not indicated on the graphs.

The moisture contents of all wood blocks uplifted during this soil burial study were in excess of the fibre saturation point (between 24 and 30%w/w, Wilkinson, 1979). The moisture contents of the untreated wood blocks were always greater than those of the CCA-treated wood blocks (table 3.1.2.1). During the soil burial study the moisture content of the untreated wood blocks increased, though moisture contents of the CCA-treated wood blocks remained approximately constant, or decreased slightly (table 3.1.2.1).

A consistent and significant difference was observed in the moisture contents of the unleached and leached, CCA-treated pine blocks, with the unleached blocks always having a substantially greater moisture level on their removal from the soil (figure

3.1.2.1). The difference between the average results of the two groups was of the order of 19.1-46.6%w/w. The range of the average moisture contents was also much smaller for the leached CCA-treated group than for the comparable unleached group, at 37.1-42.6%w/w, and 37.1-83.7%w/w for the unleached blocks (figure 3.1.2.1). Furthermore, the variability within the leached CCA-treated group was less than that within the comparable unleached group, with standard deviations in the range 0.7-1.8%w/w for leached, CCA-treated pine blocks and 2.7-12.9%w/w for similar unleached blocks (table 3.1.2.1). Since the differences in moisture uptake by the unleached and leached, CCA-treated blocks were so clear-cut statistical analysis was not considered necessary. The average moisture contents of the unleached and leached, untreated pine blocks were very similar throughout the study (figure 3.1.2.1), as were their standard deviations (table 3.1.2.1).

The effect of pre-burial leaching on the subsequent moisture uptake by wood blocks during soil burial was investigated further in the second soil burial study of this experimental programme.

3.1.2.2 Weight loss of the wood blocks.

Average weight loss results and standard deviations for each replicate group of wood blocks are presented in table 3.1.2.2 and average results are shown in figure 3.1.2.2.

The time at which decay of the buried wood blocks was initiated, if decay did occur, was obtained by estimating the point at which weight loss exceeded 3% from figure 3.1.2.2. The results of these

estimates are presented in table 3.1.2.3. The rate of weight loss for each group of wood blocks was estimated using the following equation,

$$\text{rate of weight loss (\%/week)} = \frac{X}{(Y - Z)}$$

where, X is the weight loss at the final sampling time (%),
Y is the final sampling time (weeks), and
Z is the time at which decay was initiated (weeks).

From figure 3.1.2.2 it was evident that there were two very different rates of weight loss measured for the untreated lime blocks: the first, more rapid rate, taking place up to the six week sampling time, and the second rate occurring between the 6 and 12 week sampling intervals. Therefore, rates of weight loss were also calculated for these two time periods for the untreated lime blocks. The rates of weight loss are presented in table 3.1.2.4.

All untreated wood blocks had weight losses in excess of 3% by the conclusion of this 12 week soil burial study (figure 3.1.2.2). The weight losses of the untreated softwood blocks were very similar throughout this study (figure 3.1.2.2), though significant weight losses were measured in the pine blocks earlier than in the spruce blocks (table 3.1.2.3). The rate of decay of the former group were also slightly more rapid (table 3.1.2.4).

The greatest weight losses of any group of wood blocks were obtained for the untreated lime blocks (figure 3.1.2.2). Soft rot decay of these blocks also occurred very rapidly, after about 1.5 weeks of soil burial (table 3.1.2.3). While the overall rate of weight loss of the untreated lime blocks was calculated to be 6.02%/week, the rate in the 1.5-6 week period was estimated to be much higher, at 11.52%/week (table 3.1.2.4) However, in the second half of the study the rate of weight loss decreased to 1.90%/week, though this rate is still greater than those estimated for the untreated softwood blocks (figure 3.1.2.2).

Two-way analysis of variance of the weight loss data for the untreated pine blocks shows that pre-burial leaching had no significant effect on the weight loss of these blocks.

The average weight loss of all CCA-treated pine and spruce blocks was less than 3%, and generally less than 2% (table 3.1.2.2, figure 3.1.2.2). CCA-treated lime blocks had mean weight losses which were slightly in excess of the 3% level after 6 and 12 weeks of soil burial, thus microbial decay of these blocks was estimated to have commenced after about 4 weeks of soil burial. However, no surface darkening, indicative of microbial decay, was apparent on these blocks. Therefore the commencement of microbial degradation of these heavily treated blocks after only 4 weeks in contact with soil was considered unlikely. Consequently no rate of decay was estimated for these blocks.

3.1.2.3 Wood block nitrogen contents.

The nitrogen contents of all blocks emplaced in soil as part of the burial experiment were determined; the results are presented in table 3.1.2.5 as mean values and standard deviations. Nitrogen data for the corresponding unburied blocks of all variables, previously presented in section 3.1.1.5, are also given in this table. Average levels are shown in figure 3.1.2.3. The nitrogen contents of the buried wood blocks at the time that soft rot decay was initiated was determined using figure 3.1.2.3 and table 3.1.2.3; the results are presented in table 3.1.2.6.

To determine whether there had been a significant increase in wood nitrogen contents during soil burial one-way analysis of variance was carried out for each wood species and preservative treatment (see section 2.6). Statistical analysis was carried out on

the wood nitrogen data including data for the unburied wood blocks and also excluding this data. The results are presented in table 3.1.2.7. Two-way analysis of variance of the pine data was used to assess the effect of pre-burial leaching on subsequent nitrogen increases during soil burial.

The nitrogen contents of all untreated wood blocks increased during the study (figure 3.1.2.3), and all increases were highly significant ($p < 0.5\%$, table 3.1.2.7). The greatest increases in wood nitrogen contents were measured in the untreated lime blocks, which also had the greatest initial nitrogen contents (figure 3.1.2.3; table 3.1.2.5). The nitrogen contents of the untreated wood blocks at the time at which significant weight loss was considered to have occurred were greater for all wood species than were those of the corresponding unburied blocks (table 3.1.2.6). Statistical analysis indicated that pre-burial leaching of untreated pine blocks has no effect on subsequent nitrogen increases during soil burial.

The average nitrogen contents of all buried, CCA-treated wood blocks were greater than for unburied blocks (figure 3.1.2.3). The increase was highly significant in the case of the softwood blocks ($p < 0.5\%$), though the significance of the increase for the CCA-treated lime blocks was not as great ($p < 5\%$, table 3.1.2.7). Figure 3.1.2.3 shows most of the increases in the wood nitrogen contents of the treated blocks occurred within the first 3 weeks of the study. When the unburied wood nitrogen contents were omitted from the statistical analysis, the degree of significance was reduced in most cases (table 3.1.2.7). This confirmed that most of the increase occurred within the first 3 weeks of soil burial. All increases in nitrogen contents of the CCA-treated wood blocks during this soil burial study were small in comparison with the untreated blocks (figure 3.1.2.3).

3.1.2.4 Preservative metal contents of the untreated and CCA-treated wood blocks during the soil burial study.

The average weight of the individual wood blocks, volume of preservative taken up by each block after impregnation and volume of preservative taken up per gramme of wood are given in table 3.1.2.8. Average results were calculated using the data on all blocks treated at the same time, i.e. those of CCA leaching experiment 1 and soil burial study 1. Thus 48 pine blocks were treated with each preservative, while for spruce and lime only 30 blocks were treated.

The preservative metal contents of all CCA-treated blocks were determined from the volume of preservative solution taken up by each wood block (liquid uptake data) and by chemical analyses. Results of these determinations are given in tables 3.1.2.9-3.1.2.11 (liquid uptake) and 3.1.2.12-3.1.2.14 (analysis). The comparable data for the unburied blocks (previously presented in tables 3.1.1.1-3.1.1.4) are included. Results of the analytical determinations are also given in figures 3.1.2.4-3.1.2.7. Copper and chromium contents of untreated wood blocks are presented in tables 3.1.2.9 and 3.1.2.10. Arsenic contents of these blocks were not determined. Possible changes in levels of the preservative metals in untreated and CCA-treated wood blocks during this soil burial study were investigated statistically using a one-way analysis of variance; the results are presented in table 3.1.2.15. The data for the unleached and leached pine blocks were compared using a two-way analysis of variance (see section 2.6).

CCA-treated spruce blocks had the greatest preservative metal concentrations, expressed as a %w/w of the wood blocks of all 3 wood species (figures 3.1.2.4-3.1.2.7). The reason for this is evident

from the data presented in table 3.1.2.8. During preservative treatment blocks of all 3 wood species took up approximately 1.9cm of solution. Therefore, if the preservative retention had been calculated on the basis of the volume of wood, levels in blocks of the 3 wood species treated with the same concentrations of CCA would have been similar. However, blocks of the different wood species, while of the same size, had different initial dry weights (table 3.1.2.8). Blocks of lime were the heaviest and those of spruce were the lightest. Therefore, when the preservative levels were expressed on the basis of the initial dry weight of the blocks prior, preservative metal concentrations in the wood are in the order, spruce > pine > lime.

Statistical analysis usually indicated no significant changes in the levels of any of the preservative metals in the CCA-treated wood blocks occurred during this study (table 3.1.2.15). However, in some cases statistical analysis did indicate a significant decrease in one of the preservative metals, and these cases will now be reviewed.

A decrease in the copper concentration of the leached, 5%w/v CCA-treated pine blocks is evident in figure 3.1.2.5. When these results were analysed statistically a highly significant decrease ($p < 0.5\%$), in the copper content was found (table 3.1.2.15). Comparing the average copper contents of the leached, unburied 5%w/v CCA-treated pine blocks with those of comparable blocks buried in soil for 12 weeks (table 3.1.2.9) indicates a decrease in copper content of about 17% occurred. However, it should be noted that the leached, unburied 5%w/v CCA-treated pine blocks had the greatest average copper contents of any replicate group of 5%w/v CCA-treated pine blocks in this experiment (table 3.1.2.9). Furthermore, in CCA leaching experiment 1 statistical analysis indicated that this same group of blocks had significantly greater copper contents than their

unleached counterparts (table 3.1.1.5).

There was a slightly significant decrease ($p < 5\%$) in the arsenic contents of the 5%w/v CCA-treated pine blocks during this study (table 3.1.2.15). Comparing the average arsenic contents of the unburied blocks and of those buried for 12 weeks (table 3.1.2.11), shows a decrease in arsenic concentration of about 12%.

A slightly significant ($p < 5\%$) decrease in the copper contents of the 3%w/v CCA-treated spruce blocks was indicated (table 3.1.2.15). Comparing the average copper concentrations in the unburied, 3%w/v CCA-treated spruce blocks and the corresponding blocks sacrificed after 12 weeks of soil burial (table 3.1.2.9) shows an 18% decrease in the copper content of the blocks during this study.

A decrease in the arsenic contents of the 3%w/v CCA-treated lime blocks during this study can be seen in figure 3.1.2.6; this decrease was significant ($p < 5\%$, table 3.1.2.15). A comparison of the relevant average values (table 3.1.2.11) shows a decrease of about 30% in the arsenic contents of the 3%w/v CCA-treated lime blocks by the end of this study.

There were no significant changes in the levels of copper and chromium in untreated pine and lime blocks during this experiment (table 3.1.2.15). One-way analysis of variance indicated highly significant changes ($p < 0.5\%$) in the levels of copper and chromium in the untreated spruce blocks occurred during the study (table 3.1.2.15). While the average copper and chromium contents of the buried spruce blocks were always greater than those of their unburied counterparts, the greatest levels of both metals were measured in the replicate group of blocks uplifted after 3 weeks of burial (tables

3.1.2.12 and 3.1.2.13). Therefore, it appears that these unusually high metal concentrations are responsible for the statistical result obtained.

Two-way analysis of variance of the pine preservative metal contents indicated that pre-burial leaching had no effect on the metal contents of the untreated and 3%w/v CCA-treated blocks during soil burial (table 3.1.2.16). Leaching produced a slightly significant difference in the copper and chromium contents of the 5%w/v CCA-treated pine blocks. The average chromium contents of the leached blocks were generally slightly greater than those of the unleached blocks (table 3.1.2.13). A similar result was obtained when a T-test was used to compare these two groups in the cold-water leach study (section 3.1.1.2).

As well as indicating that leaching affected the copper contents of the 5%w/v CCA-treated pine, slightly significant ($p < 5\%$) interaction mean squares were obtained for both the copper and arsenic results for the unleached and leached, 5%w/v CCA-treated pine blocks (table 3.1.2.16), implying that the two groups are behaving differently. Leached, unburied 5%w/v CCA-treated pine blocks had the greatest average copper and arsenic contents, determined by analysis, of any 5%w/v CCA-treated pine blocks in this experiment (tables 3.1.2.12 and 3.1.2.14). Furthermore the copper and arsenic contents of all leached, buried, 5%w/v CCA-treated pine blocks were lower than those of the corresponding unburied blocks, though such a difference was not found for the unleached, 5%w/v CCA-treated pine blocks (table 3.1.2.15). Therefore, it is not surprising that significant ($p < 5\%$) interaction mean squares were obtained when these results were compared (table 3.1.2.16).

3.1.2.5 Comparative study of methods for the determination of preservative metal levels in soil.

Introduction.

Three methods were compared for the determination of copper, chromium and arsenic concentrations in soil (see section 2.4.1). The results from each method are presented in tables 3.1.2.17-3.1.2.21.

A known amount of CCA was added to some soil samples prior to the determinations (see section 2.4). These known amounts, expressed on the basis of the dry weight of the soil samples, are shown along with the relevant results table. To assess the relative efficiency of the different techniques, the percentage recovery of the added metals was calculated as follows,

$$\text{Recovery (\%)} = \frac{(B - A)}{C} * 100$$

where, A is the average metal concentration of the untreated soil sample,
B is the average metal concentration of the soil+CCA sample,
and C is the estimated additional concentration of metal based on the amount of CCA added and the known weight of soil to which it was added.

The results of these calculations are presented in tables 3.1.2.17-3.1.2.21.

Method 1. Sulphuric acid (concentrated) / hydrogen peroxide digest.

When method 1 was first carried out good recoveries of added copper and chromium were obtained (table 3.1.2.17). Arsenic results for both soil types were very variable (table 3.1.2.17). While a comparison of the average arsenic concentrations shows a 66% recovery of added arsenic, the size of the standard deviations must be borne in mind in technique selection.

When the technique was repeated, copper concentrations obtained

for the untreated soil samples were significantly greater than those obtained formerly (table 3.1.2.17), though chromium concentrations were similar. Recoveries of added copper and chromium were reduced in comparison to levels previously obtained (table 3.1.2.17). Arsenic concentration determinations were not carried out on this occasion.

Method 2. i. EDTA leach - disodium salt at pH 9.

Concentrations of all three preservative metals were far lower when determined by this technique (table 3.1.2.18) in comparison to method 1 (table 3.1.2.17). Recoveries were also very poor (table 3.1.2.17), particularly of arsenic. It was considered that this could be due to the high concentration of sodium in the solutions which were run through the AAS. Therefore the technique was repeated using the ammonium salt of EDTA.

ii. EDTA leach - ammonium salt at pH 7.

There was very little difference between the copper and chromium concentrations determined for the untreated soil using the ammonium salt at pH 7 (table 3.1.2.19) and the disodium salt at pH 9 (table 3.1.2.18). Soil arsenic concentrations were not determined in this case. Improved percentage recoveries of copper were obtained using the ammonium salt at pH7 (table 3.1.2.19), in comparison with the recovery of copper when the disodium salt was utilised. The level of recovery was reasonably consistent, irrespective of the estimated amount of CCA added. However, the recovery of added chromium was very poor (table 3.1.2.19). This increased slightly with increasing levels of added chromium, though it never exceeded 40%.

iii. EDTA leach - ammonium salt at pH 9.

Method 2 (ii) was repeated with the pH of the leaching solution adjusted to pH 9 with ammonium hydroxide solution. The results of this determination are presented in table 3.1.2.20. Slightly higher background copper concentrations were obtained using the ammonium salt at pH 9 (table 3.1.2.20) than had been determined for either of the previous two EDTA leaching techniques (tables 3.1.2.18 and 3.1.2.19), though the average chromium concentration was very similar in all three cases. The estimated recovery of added copper using the ammonium salt of EDTA at pH 9 was the best obtained for any EDTA leaching technique (tables 3.1.2.18-3.1.2.20). The percentage recovery of chromium was also generally improved at around 40% (table 3.1.2.20), though one very low value of 14% was obtained.

Arsenic concentrations of the soil were not determined using this technique.

Method 3. - Sulphuric acid (2.5M) / hydrogen peroxide leach.

This technique was carried out three times, with different levels of CCA being added to the test soil on each occasion. Results of these determinations are presented in table 3.1.2.21. Background concentrations of copper, chromium and arsenic were greater when determined by this technique (table 3.1.2.21) than obtained for any of the EDTA leaching techniques (tables 3.1.2.18-3.1.2.20), though they were all lower than concentrations obtained using method 1 (table 3.1.2.17). Concentrations of copper in the untreated soil determined at three different times were generally similar (table 3.1.2.21), though the chromium concentrations exhibited some variability.

Percentage recoveries of added copper and chromium were good (table 3.1.2.21) and appeared to be independent of the amount of metal added. Recoveries of arsenic were not very high (table 3.1.2.21). However, while the arsenic concentrations determined by this technique had relatively large standard deviations (table 3.1.2.18), as in method 1 (table 3.1.2.14), there was a much clearer difference between the background and experimental levels of arsenic when method 3 was used.

Discussion.

In general the leaching techniques (methods 2 and 3) were found to give more reproducible results and were less time-consuming than the acid digest (method 1). However, while leaching the soil samples with a solution of the ammonium salt of EDTA at pH 9 (method 2 (ii), table 3.1.2.20), gave good recoveries of copper, recoveries of added chromium and arsenic were generally poor when the EDTA salts were used. Leaching of soil samples with sulphuric acid and hydrogen peroxide gave reproducible background metal levels and good recoveries of added copper and chromium (table 3.1.2.21). While the recovery of arsenic was not particularly good using this method, the relatively low variability of the arsenic data obtained made it superior, in this respect, to method 1. For these reasons, method 3, the sulphuric acid (2.5M)/hydrogen peroxide leach was chosen for the determination of copper, chromium and arsenic concentrations in soil samples during the work reported here.

3.1.2.6 Preservative metal levels in soil adjacent to untreated and CCA-treated wood blocks during the soil burial study.

Copper, chromium and arsenic concentrations (ug g^{-1} soil [dry weight]), of the soil used in this study were determined to be 41.2 ± 2.7, 33.1 ± 2.2 and 23.6 ± 26.7 respectively immediately prior to the setting up of the experiment. These results were used in statistical analyses carried out in this section; the average results are indicated on the appropriate graphs as the zero time data. The preservative metal concentrations of soil collected from adjacent to all wood blocks during this soil burial study are given in tables 3.1.2.22-3.1.2.24, and the average results are presented in figures 3.1.2.8-3.1.2.10.

To determine whether there had been a statistically significant change in the preservative metal concentrations in soil adjacent to the buried wood blocks, one-way analysis of variance was carried out on the data (see section 2.6). The results of these statistical analysis are presented in table 3.1.2.25.

To further statistically assess any differences in concentrations of preservative metals in the soil collected from around the buried wood blocks, two-way analysis of variance was carried out. This allowed a comparison of the metal concentrations of soil collected from adjacent to untreated wood blocks of each wood species with data for soil from around CCA-treated blocks of the same wood species. Data for soil which had been adjacent to 3 and 5%w/v CCA-treated wood blocks were similarly analysed, as were results for unleached and leached pine blocks. Results of these analyses are presented in tables 3.1.2.26 and 3.1.2.27.

In all cases the copper contents of soil which had been in

contact with the CCA-treated blocks were greater than those from around the untreated blocks (figure 3.1.2.8). Two-way analysis of variance confirmed these differences to be significant (table 3.1.2.26). In general, most of the increase in soil copper contents occurred within 3 weeks of placing the blocks in soil (figure 3.1.2.8). The average copper content of samples continued to increase with time and all increases were significant ($p < 0.5\%$, table 3.1.2.25). The greatest increases in soil copper concentration were measured in samples collected from around CCA-treated lime blocks (figure 3.1.2.8).

A significant ($p < 0.5\%$) change in the copper level of soil collected from around pine untreated blocks was indicated (figure 3.1.2.25). Figure 3.1.2.8 shows that a decrease in the average copper content of soil in contact with these blocks occurred with time. This was the only case in which any significant change in levels of any of the three preservative metals occurred in soil adjacent to untreated blocks (table 3.1.2.25).

Curves of the average copper contents of soil collected from around 3 and 5%w/v CCA-treated blocks appeared to be very similar for each wood species (figure 3.1.2.8). Two-way analyses of variance were carried out to compare the soil metal data for the two preservative treating levels. These analyses confirmed that in all cases the level of treatment had no significant effect on the copper content of the adjacent soil (table 3.1.2.25).

The soil copper contents obtained for the leached, CCA-treated pine samples were consistently lower than those of their unleached counterparts (figure 3.1.2.8, table 3.1.2.19); this difference was found to be significant (table 3.1.2.25).

There were no apparent changes in the chromium levels of soil adjacent to untreated wood blocks and to CCA-treated softwood blocks during this experiment (figure 3.1.2.9). With the exception of soil collected from around the unleached, 3%w/v CCA-treated pine blocks, no significant change in the chromium levels of any of these soil samples was measured (table 3.1.2.25). In the case of soil adjacent to unleached, 3%w/v CCA-treated pine blocks, the average chromium concentrations were consistently greater than the initial value (figure 3.1.2.9), though the difference was small. Other average soil chromium concentrations of soil around untreated and CCA-treated softwood blocks were greater than the levels measured for these pine samples (table 3.1.2.23), though no significant change was indicated in these cases (table 3.1.2.25). This is due to the larger standard deviations which were also obtained in the majority of cases (table 3.1.2.23).

Highly significant ($p < 0.05\%$) increases in the chromium content of soil adjacent to CCA-treated lime blocks were measured (figure 3.1.2.9; table 3.1.2.25). Most of these increases occurred in the first 3 weeks of the study (figure 3.1.2.9). Two-way analysis of variance was carried out on the lime soil chromium results. This confirmed the increase in chromium concentrations around the CCA-treated lime blocks (table 3.1.2.26). This statistical analysis also demonstrated that there was no difference between the chromium concentrations of soil adjacent to 3 and 5%w/v CCA-treated lime blocks (table 3.1.2.23), as shown in figure 3.1.2.9.

Leaching was not found to have any effect on chromium concentrations in soil around untreated and 5%w/v CCA-treated pine blocks (table 3.1.2.27). Soil chromium concentrations around unleached, 3%w/v CCA-treated pine blocks were found to be

significantly greater ($p < 5\%$), than those around the corresponding leached blocks (table 3.1.2.27), though the difference was very small (figure 3.1.2.9).

With the exception of the arsenic contents of soil adjacent to 5%w/v CCA-treated spruce blocks, all soil arsenic results for samples collected from around, untreated and CCA-treated softwood blocks were very similar (figure 3.1.2.10). One and two-way analyses of variance of the softwood arsenic data indicated no significant changes in the arsenic concentrations of any of these groups had occurred (tables 3.1.2.22 and 3.1.2.24), including 5%w/v CCA-treated spruce.

The arsenic concentrations of soil adjacent to 3 and 5%w/v CCA-treated lime blocks increased, this increase continuing throughout the soil burial period (figure 3.1.2.10). The increase in average soil arsenic contents around the 3%w/v CCA-treated lime blocks appeared to be greater than the increase around the 5%w/v CCA-treated lime blocks. While in the former case the increase was highly significant ($p < 0.5\%$), in the latter case it was only moderately significant ($p < 1\%$), (table 3.1.2.25). The relatively low statistical significance of the results obtained for the 5%w/v CCA-treated lime samples is at least partly attributable to the large standard deviations obtained for the replicate groups of soil arsenic determinations (table 3.1.2.24). Two-way analysis of variance of the CCA-treated lime soil arsenic results showed no significant difference in the arsenic concentrations of soil adjacent to 3 and 5%w/v CCA-treated lime blocks (table 3.1.2.23).

3.1.2.7 Figures 3.1.2.1-3.1.2.10.

Figure 3.1.2.1 Average moisture contents of pine blocks during the soil burial study.

Figure 3.1.2.2 Average weight losses of wood blocks during the soil burial study.

Figure 3.1.2.3 Average nitrogen contents of wood blocks during the soil burial study.

Figure 3.1.2.4 Mean preservative metal contents ± standard deviations of unleached, CCA-treated pine blocks during the soil burial study.

Figure 3.1.2.5 Mean preservative metal contents ± standard deviations of leached, CCA-treated pine blocks during the soil burial study.

Figure 3.1.2.6 Mean preservative metal contents ± standard deviations of CCA-treated spruce blocks during the soil burial study.

Figure 3.1.2.7 Mean preservative metal contents ± standard deviations of CCA-treated lime blocks during the soil burial study.

Figure 3.1.2.8 Average copper contents of soil adjacent to wood blocks during the soil burial study.

Figure 3.1.2.9 Average chromium contents of soil adjacent to wood blocks during the soil burial study.

Figure 3.1.2.10 Average arsenic contents of soil adjacent to wood blocks during the soil burial study.

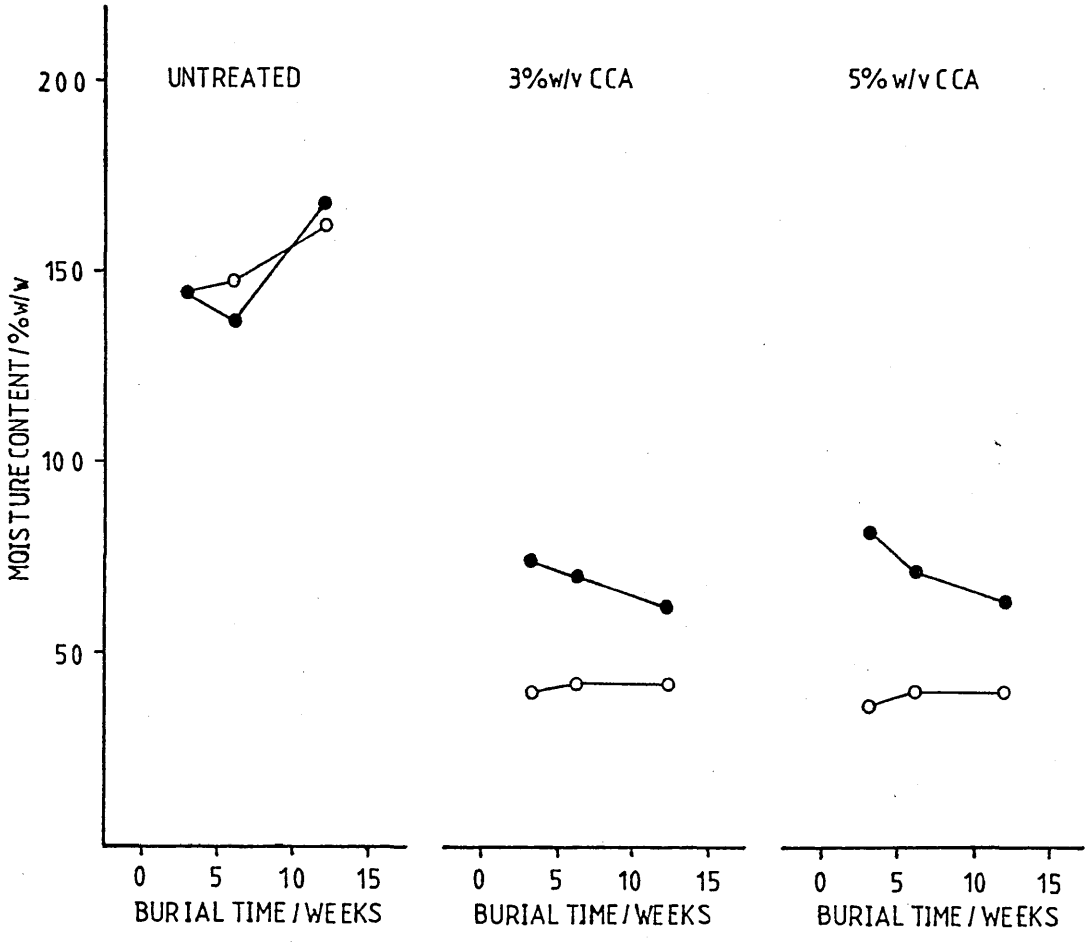


Figure 3.1.2.1 Average moisture contents of pine blocks during the soil burial study. Average is based on a minimum of 5 replicates.

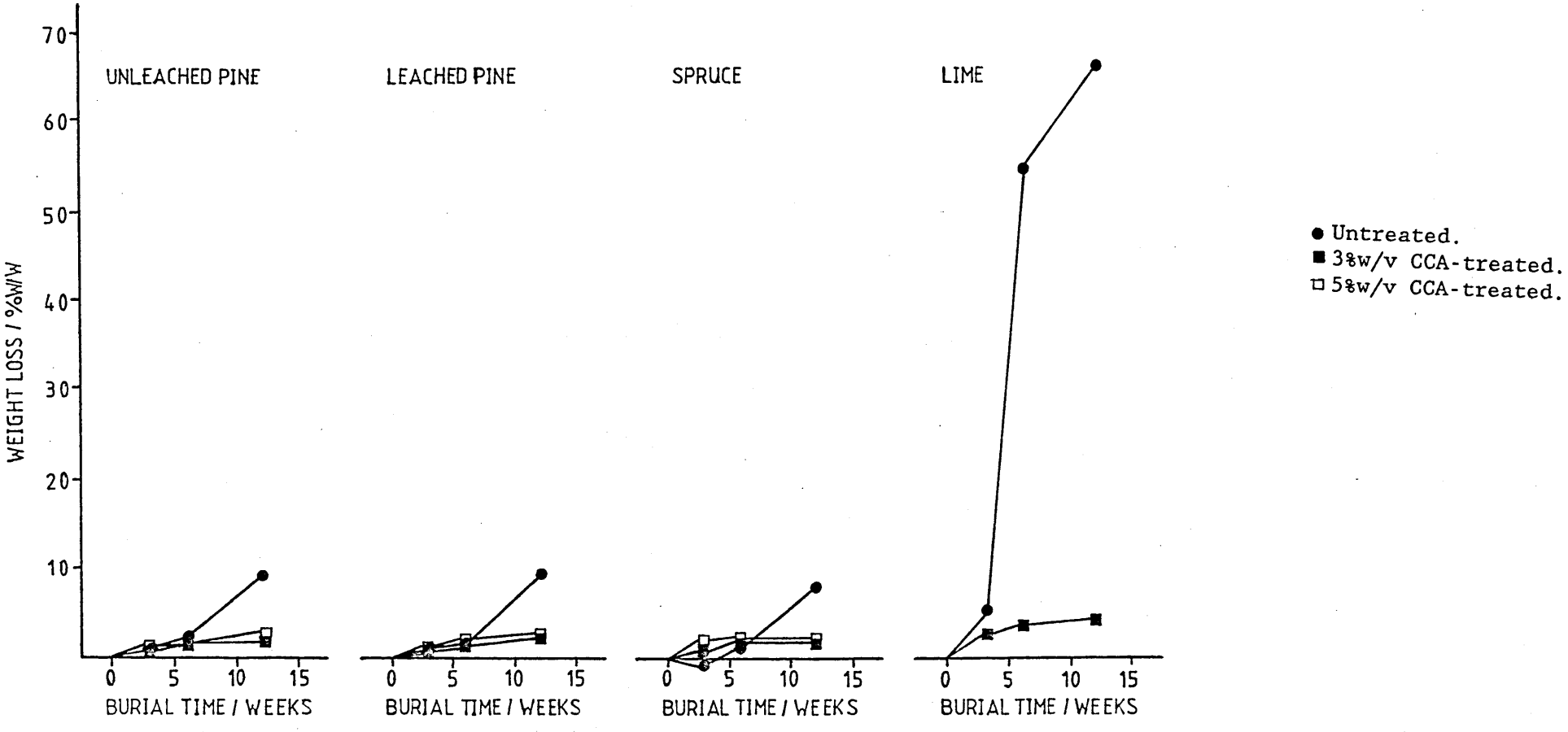


Figure 3.1.2.2 Average weight losses of wood blocks during the soil burial study. Note that the curves for the 3 and 5%w/v CCA-treated lime blocks are identical. Average is based on a minimum of 5 replicates.

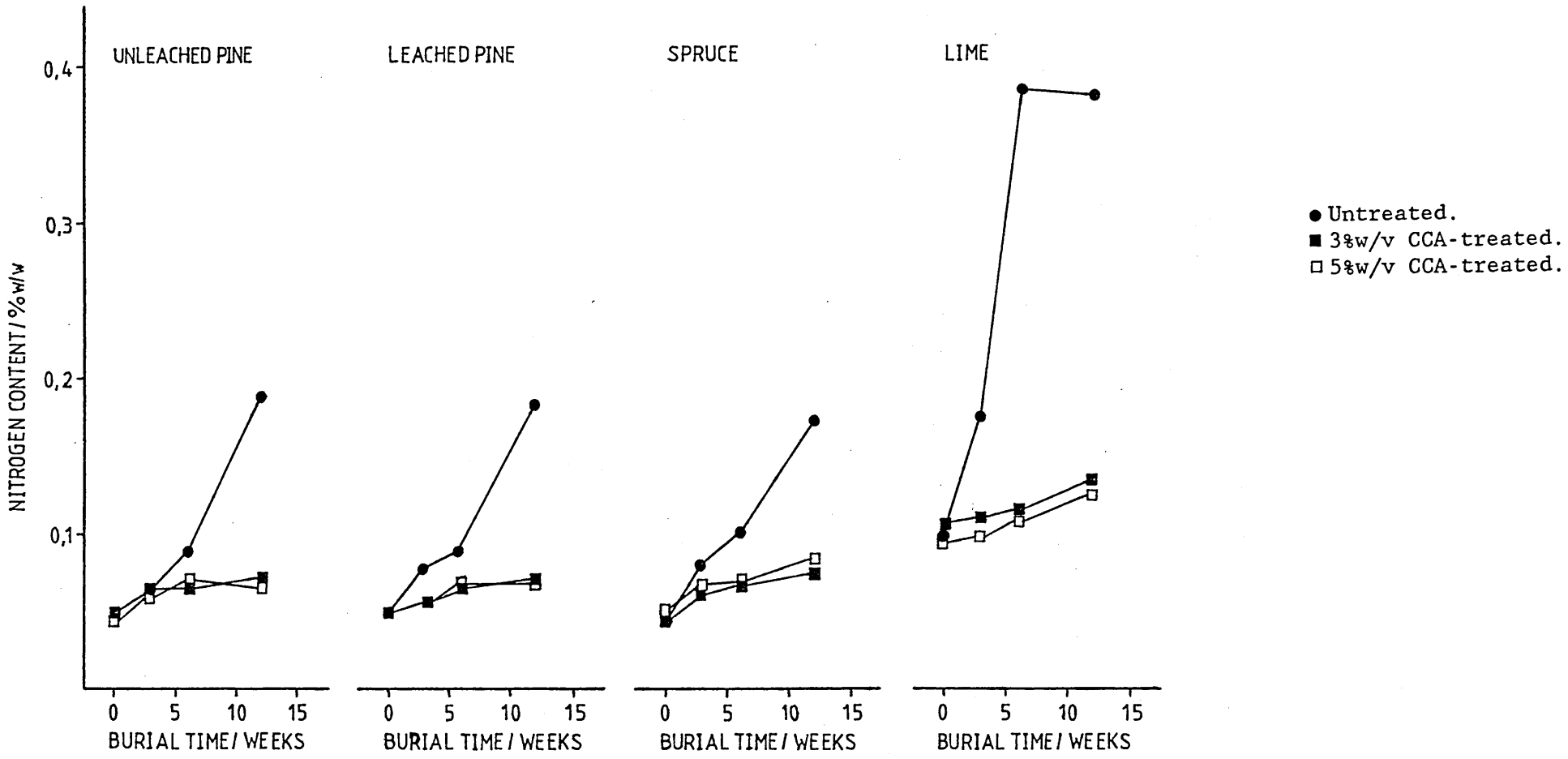


Figure 3.1.2.3 Average nitrogen contents of wood blocks during the soil burial study.

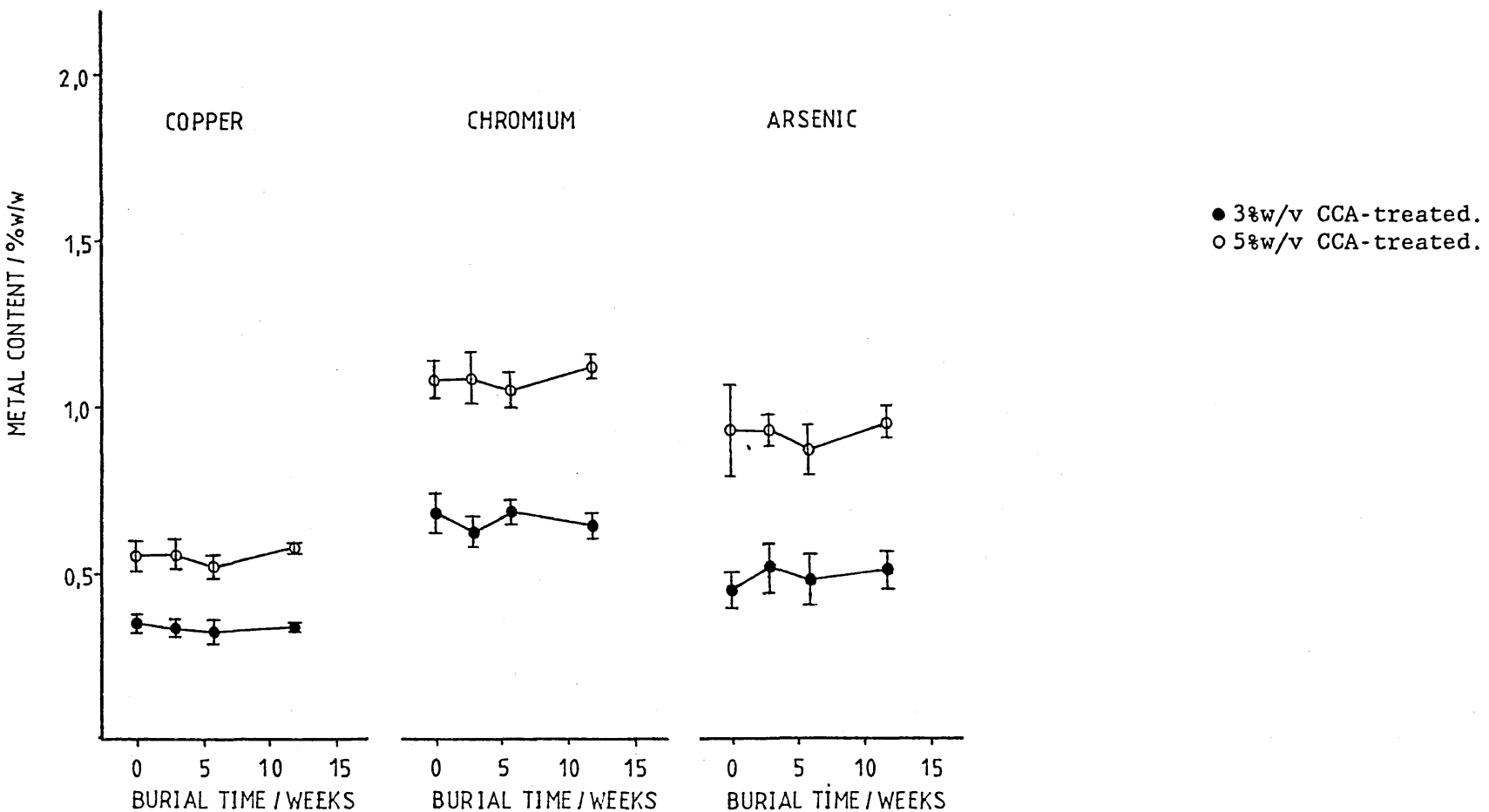


Figure 3.1.2.4 Mean preservative metal contents ± standard deviations of unleached, CCA-treated pine blocks during the soil burial study. Mean is based on a minimum of 5 replicates.

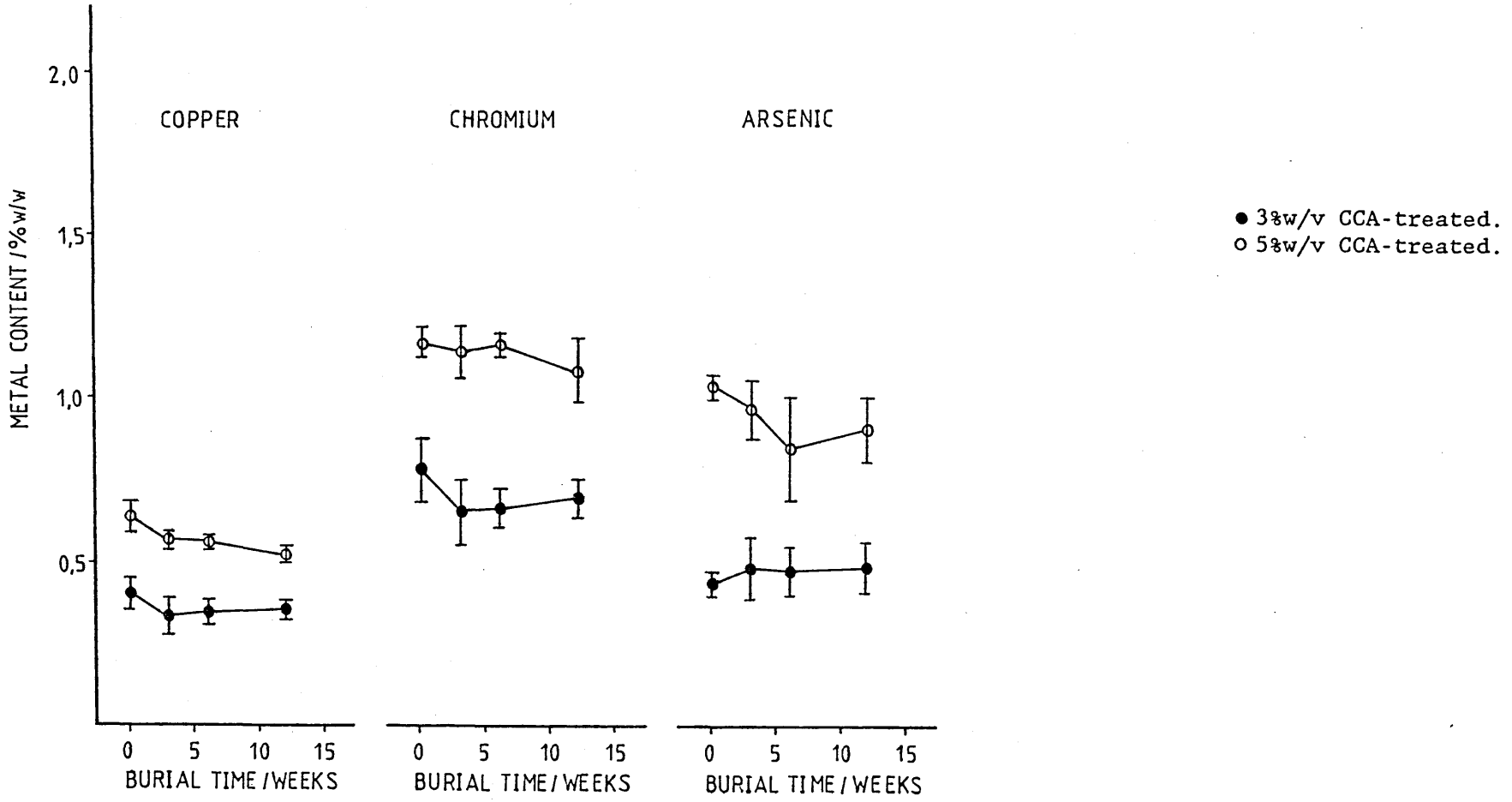


Figure 3.1.2.5 Mean preservative metal contents ± standard deviations of leached, CCA-treated pine blocks during the soil burial study. Mean is based on a minimum of 5 replicates.

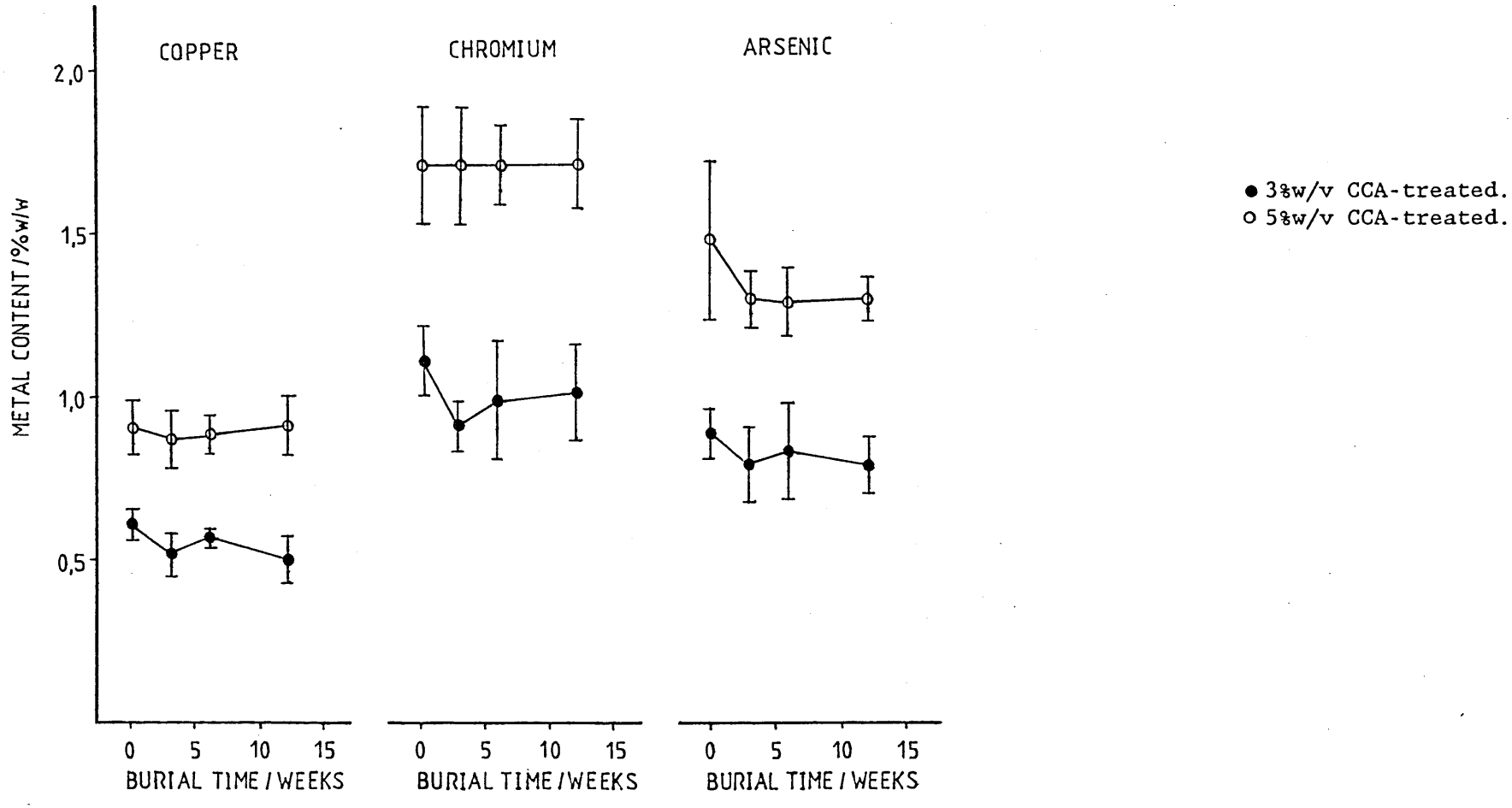


Figure 3.1.2.6 Mean preservative metal contents ± standard deviations of CCA-treated spruce blocks during the soil burial study. Mean is based on a minimum of 5 replicates.

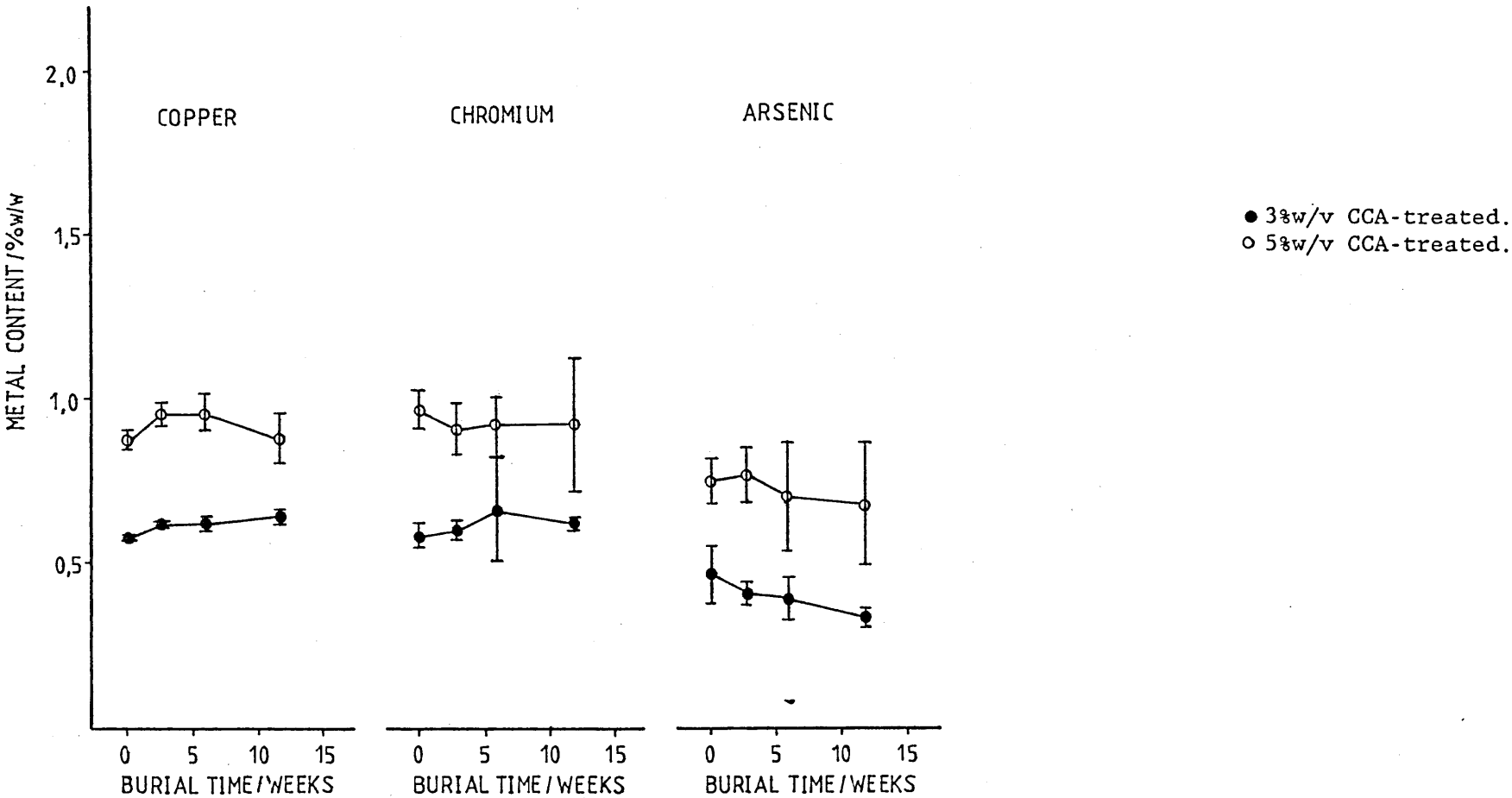


Figure 3.1.2.7 Mean preservative metal contents ± standard deviations of CCA-treated lime blocks during the soil burial study. Mean is based on a minimum of 5 replicates.

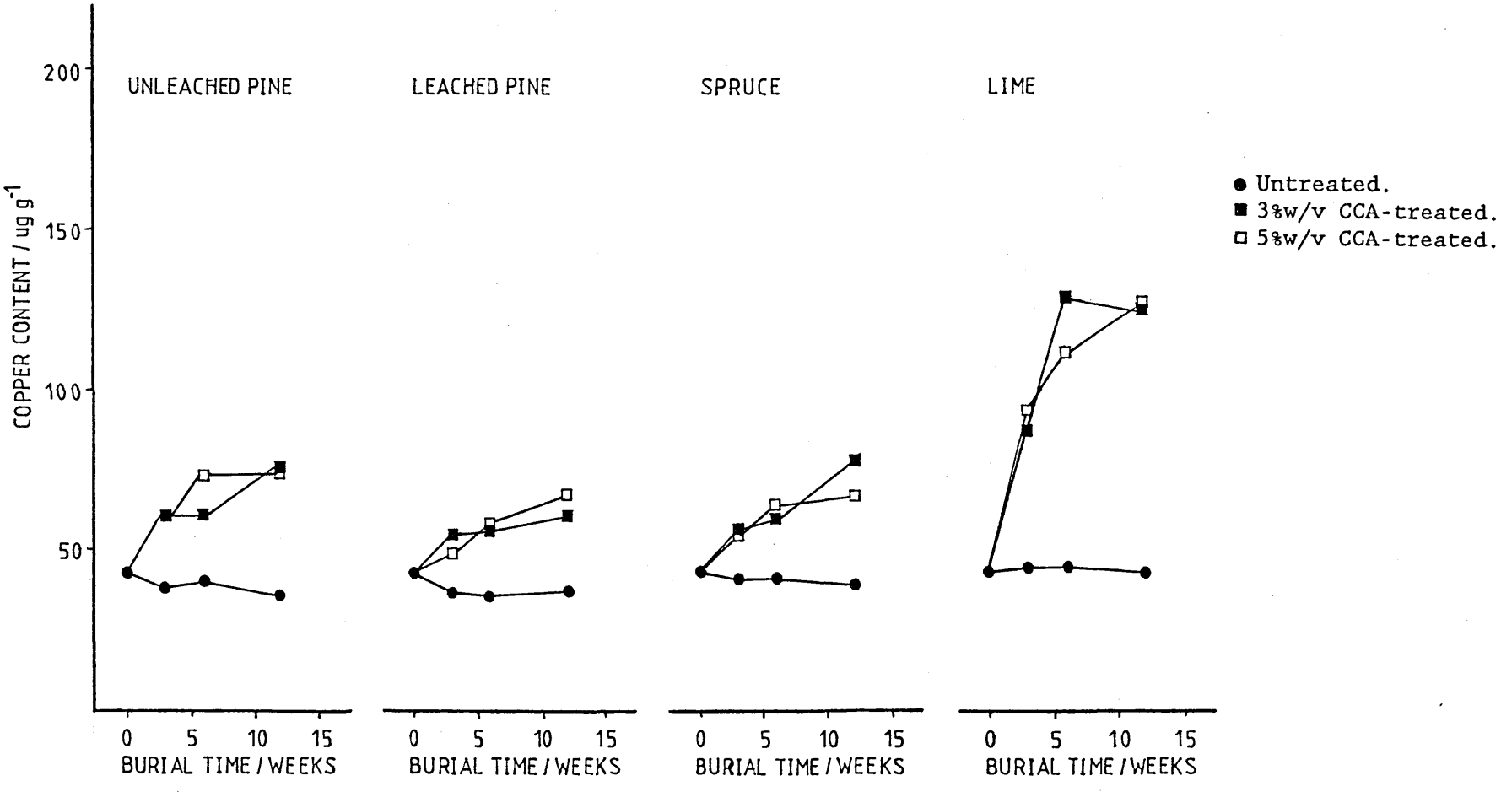


Figure 3.1.2.8 Average copper contents of soil adjacent to wood blocks during the soil burial study. Average is based on a minimum of 5 replicates.

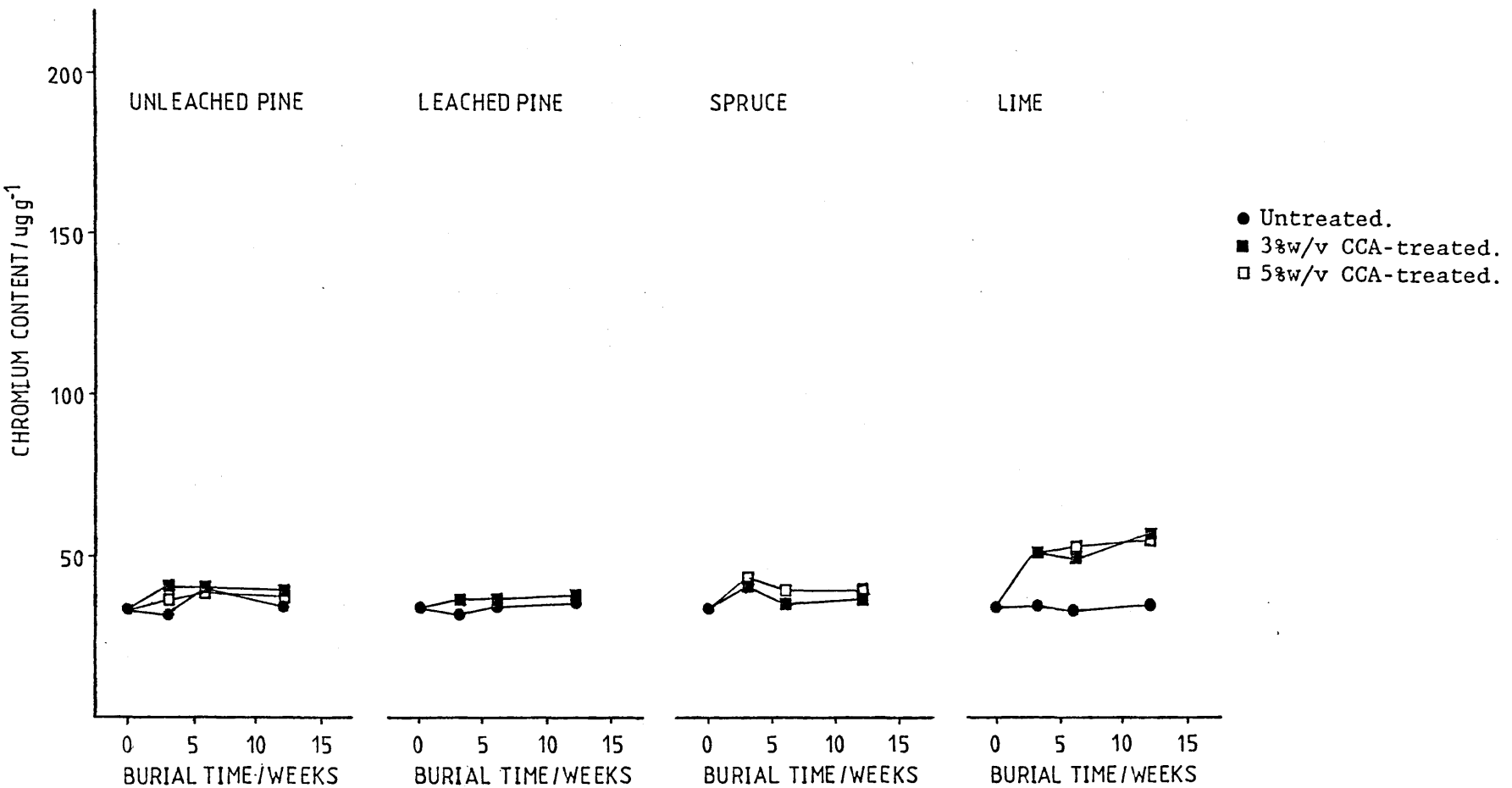


Figure 3.1.2.9 Average chromium contents of soil adjacent to wood blocks during the soil burial study. Average is based on a minimum of 5 replicates.

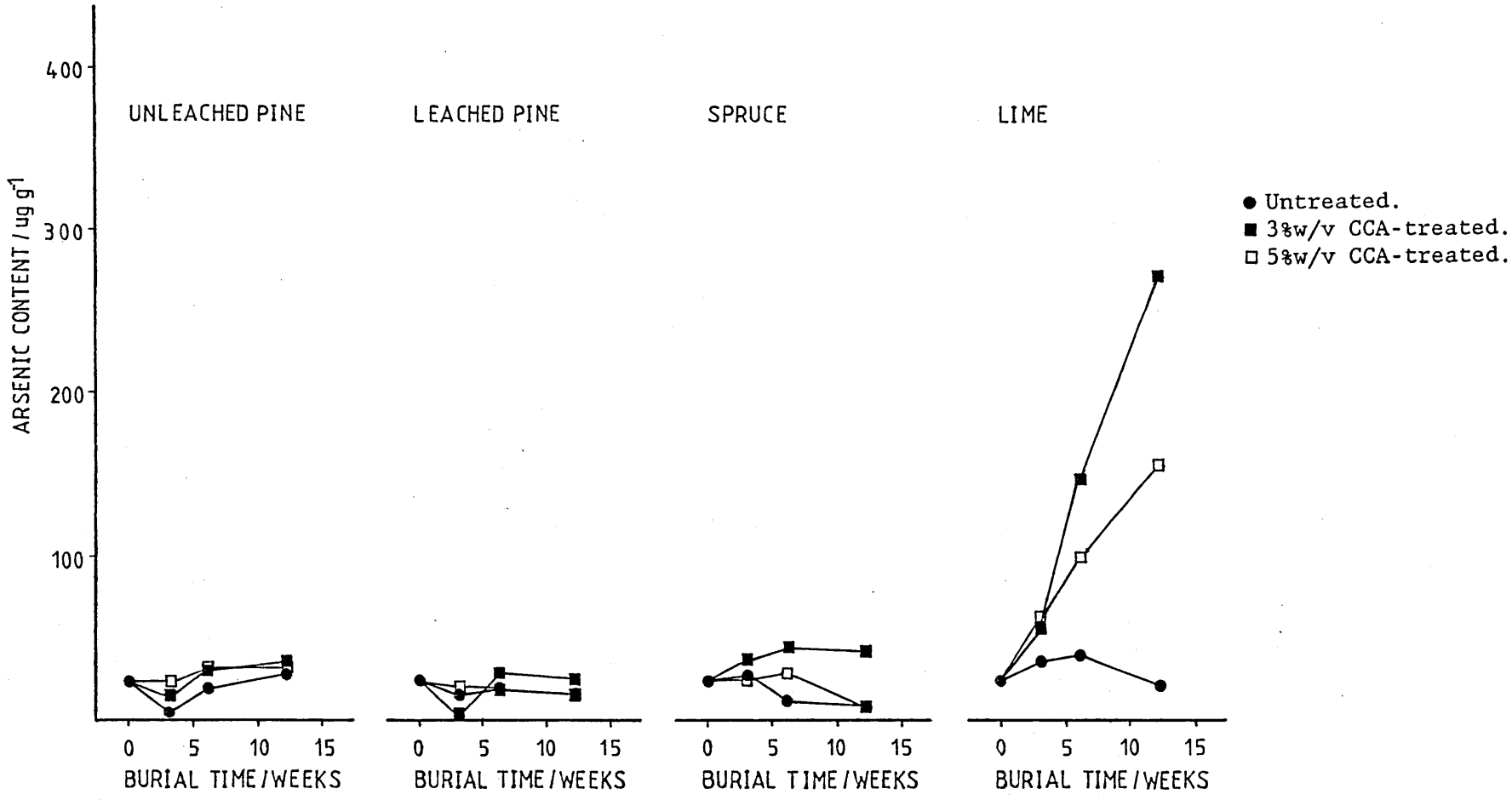


Figure 3.1.2.10 Average arsenic contents of soil adjacent to wood blocks during the soil burial study. Average is based on a minimum of 5 replicates.

3.1.2.8 Tables 3.1.2.1-3.1.2.27.

Table 3.1.2.1 Moisture contents of wood blocks during soil burial study 1, experimental programme 1.

Table 3.1.2.2 Weight losses of wood blocks during soil burial study 1, experimental programme 1.

Table 3.1.2.3 Estimated time of initiation of microbial decay.

Table 3.1.2.4 Estimated rate of decay.

Table 3.1.2.5 Nitrogen contents of wood blocks during soil burial study 1, experimental programme 1.

Table 3.1.2.6 Nitrogen contents at estimated time of initiation of microbial decay.

Table 3.1.2.7 Statistical comparison (one-way analysis of variance), of nitrogen contents data.

Table 3.1.2.8 Preservative liquid uptake after impregnation by wood blocks, along with the weight of individual wood blocks and preservative liquid uptake per gramme of wood.

Table 3.1.2.9 Copper contents, based on liquid uptake, wood blocks.

Table 3.1.2.10 Chromium contents, based on liquid uptake, of wood blocks.

Table 3.1.2.11 Arsenic contents, based on liquid uptake, of wood blocks.

Table 3.1.2.12 Copper contents of wood blocks during the soil burial study, determined by analysis.

Table 3.1.2.13 Chromium contents of wood blocks during the soil burial study, determined by analysis.

Table 3.1.2.14 Arsenic contents wood blocks during the soil burial study, determined by analysis.

Table 3.1.2.15 Results of statistical analyses to investigate possible preservative metal losses from wood blocks during the study.

Table 3.1.2.16 Results of statistical analyses to assess differences in preservative metal contents of unleached and leached pine blocks with time.

Table 3.1.2.17 Metal concentrations of soil samples determined by method 1 (see section 2.4.1).

Table 3.1.2.18 Metal concentrations of soil samples determined by method 2 (i) (see section 2.4.1).

Table 3.1.2.1 Moisture contents of wood blocks during soil burial study 1, experimental programme 1. Mean results ± standard deviations are presented (mean is based on a minimum of 5 replicates).

Wood species	Burial time (weeks)	Moisture content (%w/w)		
		Untreated	3%w/v CCA	5%w/v CCA
Pine (unleached)	3	146.3± 5.8	74.2± 8.2	83.7±12.9
	6	137.8±11.2	70.1± 3.3	72.0± 9.8
	12	167.7+15.8	61.7+ 2.7	63.4+ 6.0
Pine (leached)	3	147.0±13.4	39.2± 1.8	37.1± 0.8
	6	147.6±10.8	41.9± 0.7	40.7± 1.5
	12	161.6+16.9	42.6+ 1.3	40.9+ 1.0
Spruce	3	187.7±24.5	117.3±20.7	123.1±19.7
	6	217.1±25.8	110.6±14.9	114.0±14.7
	12	234.5+37.3	97.9+29.7	111.6+19.4
Lime	3	50.2± 2.6	47.8± 2.8	51.9± 5.9
	6	145.5±39.4	47.2± 2.2	52.9± 7.4
	12	192.1+55.9	51.8+ 2.5	59.5+ 2.9

Table 3.1.2.2 Weight losses of wood blocks during soil burial study 1, experimental programme 1. Mean results ± standard deviations are presented (mean is based on a minimum of 5 replicates).

Wood species	Burial time (weeks)	Weight loss (%)		
		Untreated	3%w/v CCA	5%w/v CCA
Pine (unleached)	3	0.90±0.46	0.83±0.32	1.12±0.49
	6	1.88±0.43	1.04±0.36	1.52±0/18
	12	9.20+0.66	1.67+0.21	2.52+0.11
Pine (leached)	3	1.13±0.20	1.01±0.51	1.44±0.63
	6	1.75±0.59	1.44±0.32	2.13±0.28
	12	8.97+1.41	1.86+0.35	2.29+0.18
Spruce	3	+0.54±0.45	0.66±0.47	1.79±0.50
	6	0.56±0.59	1.30±0.32	2.21±0.32
	12	7.42+2.37	1.69+0.62	2.56+0.45
Lime	3	5.51±3.47	2.71±0.36	2.79±0.37
	6	54.83±11.2	3.35±0.38	3.52±0.22
	12	66.26+5.68	3.92+0.25	4.13+0.28

Table 3.1.2.3 Estimated time of initiation of microbial decay (weeks).

Wood species	Treatment		
	Untreated	3%w/v CCA	5%w/v CCA
Pine (unleached)	6.50	<3%	<3%
Pine (leached)	6.75	<3%	<3%
Spruce	7.75	<3%	<3%
Lime	1.50	4(*)	4(*)

Key. <3% mean weight loss did not exceed 3% throughout this soil burial study.
(*) weight loss exceeded 3%, but considered unlikely to be due to microbial decay.

Table 3.1.2.4 Estimated rate of decay (%/week).

Pine (unleached)	Pine (leached)	Spruce	Lime
1.13	1.14	1.04	6.02 a. 11.52 b. 1.90

Key. a. rate of decay in period 1.50-6 weeks.
b. rate of decay in period 6-12 weeks.

Table 3.1.2.5 Nitrogen contents of wood blocks during soil burial study 1, experimental programme 1. Mean results ± standard deviations are presented (mean is based on a minimum of 5 replicates).

Wood species	Burial time (weeks)	Nitrogen content (%w/w)		
		Untreated	3%w/v CCA	5%w/v CCA
Pine (unleached)	Unburied	0.049±0.005	0.049±0.005	0.045±0.002
	3	0.063±0.003	0.062±0.003	0.065±0.006
	6	0.088±0.009	0.065±0.006	0.071±0.004
	12	0.189+0.024	0.071+0.007	0.064+0.015
Pine (leached)	Unburied	0.047±0.006	0.048±0.006	0.049±0.005
	3	0.075±0.005	0.055±0.004	0.057±0.003
	6	0.088±0.006	0.063±0.006	0.067±0.007
	12	0.185+0.028	0.067+0.006	0.067+0.008
Spruce	Unburied	0.046±0.005	0.047±0.008	0.054±0.004
	3	0.080±0.013	0.061±0.010	0.067±0.004
	6	0.099±0.015	0.066±0.006	0.067±0.008
	12	0.173+0.028	0.074+0.007	0.085+0.015
Lime	Unburied	0.098±0.029	0.106±0.019	0.093±0.017
	3	0.176±0.032	0.112±0.009	0.115±0.010
	6	0.388±0.041	0.116±0.009	0.107±0.012
	12	0.385+0.036	0.134+0.019	0.124+0.017

Table 3.1.2.6 Nitrogen contents (%w/w) at estimated time of initiation of microbial decay (weeks).

Pine (unleached)	Pine (leached)	Spruce	Lime
0.100	0.100	0.124	0.138

Table 3.1.2.7 Statistical comparison (one-way analysis of variance), of nitrogen contents data of each wood species and preservative treatment during soil burial study 1, experimental programme 1; A. including data for unburied wood blocks, B. excluding data for unburied wood blocks.

Wood species	Untreated		3%w/v CCA treated		5%w/v CCA treated	
	A	B	A	B	A	B
Pine (unleached)	***	***	***	*	***	NS
Pine (leached)	***	***	***	**	***	*
Spruce	***	***	***	*	***	***
Lime	***	***	*	*	*	NS

Key. NS No significant difference.
* Significant difference: probability of difference arising by chance is < 5%
** Significant difference: probability of difference arising by chance is < 1%
*** Significant difference: probability of difference arising by chance is < 0.5%

Table 3.1.2.8 Preservative liquid uptake after impregnation by wood blocks (soil burial study 1, experimental programme 1), along with the weight of individual wood blocks and preservative liquid uptake per gramme of wood. Mean results ± standard deviations are presented (for pine, n=48, for lime and spruce n=30).

Wood species	Preservative concentration	Liquid uptake per block (cm^3)	Weight of block (g)	Liquid uptake per g wood ($cm^3 g^{-1}$)
Pine	3%	1.9589±0.1548	1.4227±0.1230	1.3800±0.0786
	5%	1.9463±0.1292	1.4260±0.1017	1.3675±0.0745
Spruce	3%	1.8780±0.2348	0.9363±0.0483	2.0083±0.2512
	5%	1.8964±0.2378	0.9258±0.0704	2.0507±0.2320
Lime	3%	1.9307±0.1395	1.6622±0.1476	1.1643±0.0549
	5%	1.8268±0.1189	1.6428±0.1169	1.1138±0.0543

Table 3.1.2.9 Copper contents, based on liquid uptake, of unburied wood blocks, and of comparable blocks intended for soil burial study 1, experimental programme 1. Mean results ± standard deviations are presented (mean is based on a minimum of 5 replicates).

Wood species	Burial time (weeks)	Copper content (%w/w)	
		3%w/v CCA	5%w/v CCA
Pine (unleached)	Unburied	0.347±0.027	0.580±0.018
	3	0.360±0.014	0.563±0.029
	6	0.348±0.015	0.561±0.044
	12	0.369+0.011	0.621+0.027
Pine (leached)	Unburied	0.359±0.014	0.605±0.015
	3	0.362±0.020	0.594±0.028
	6	0.356±0.021	0.585±0.015
	12	0.357+0.034	0.563+0.037
Spruce	Unburied	0.558±0.068	0.932±0.077
	3	0.480±0.069	0.863±0.094
	6	0.532±0.053	0.887±0.060
	12	0.485+0.079	0.877+0.098
Lime	Unburied	0.299±0.013	0.469±0.042
	3	0.300±0.012	0.473±0.013
	6	0.296±0.010	0.475±0.016
	12	0.298+0.014	0.480+0.022

Table 3.1.2.10 Chromium contents, based on liquid uptake, of unburied wood blocks, and of comparable blocks intended for soil burial study 1 experimental programme 1. Mean results ± standard deviations are presented (mean is based on a minimum of 5 replicates).

Wood species	Burial time (weeks)	Chromium content (%w/w)	
		3%w/v CCA	5%w/v CCA
Pine (unleached)	Unburied	0.656±0.050	1.098±0.035
	3	0.680±0.026	1.067±0.055
	6	0.659±0.029	1.062±0.084
	12	0.699+0.022	1.108+0.049
Pine (leached)	Unburied	0.680±0.026	1.146±0.028
	3	0.685±0.037	1.125±0.054
	6	0.673±0.039	1.109±0.029
	12	0.676+0.064	1.077+0.059
Spruce	Unburied	1.060±0.132	1.766±0.146
	3	0.908±0.131	1.635±0.177
	6	1.007±0.101	1.681±0.113
	12	0.942+0.179	1.622+0.185
Lime	Unburied	0.566±0.025	0.888±0.079
	3	0.568±0.024	0.897±0.024
	6	0.560±0.020	0.900±0.031
	12	0.565+0.027	0.893+0.041

Table 3.1.2.11 Arsenic contents, based on liquid uptake, of unburied wood blocks, and of comparable blocks intended for soil burial study 1, experimental programme 1. Mean results ± standard deviations are presented (mean is based on a minimum of 5 replicates).

Wood species	Burial time (weeks)	Arsenic content (%w/w)	
		3%w/v CCA	5%w/v CCA
Pine (unleached)	Unburied	0.452±0.035	0.761±0.024
	3	0.456±0.018	0.739±0.038
	6	0.454±0.020	0.736±0.058
	12	0.481+0.015	0.781+0.035
Pine (leached)	Unburied	0.468±0.018	0.794±0.099
	3	0.472±0.026	0.780±0.037
	6	0.463±0.027	0.768±0.020
	12	0.465+0.044	0.739+0.048
Spruce	Unburied	0.727±0.089	1.223±0.101
	3	0.626±0.091	1.132±0.123
	6	0.694±0.070	1.164±0.078
	12	0.633+0.103	1.151+0.128
Lime	Unburied	0.390±0.017	0.615±0.055
	3	0.392±0.016	0.627±0.024
	6	0.386±0.014	0.624±0.022
	12	0.383+0.032	0.630+0.029

Table 3.1.2.12 Copper contents of wood blocks during the soil burial study (experimental programme 1), determined by analysis. Mean results ± standard deviations are presented (mean is based on a minimum of 5 replicates).

Wood species	Burial time (weeks)	Copper content (%w/w)		
		Untreated	3%w/v CCA	5%w/v CCA
Pine (unleached)	Unburied	0.009±0.002	0.350±0.032	0.552±0.048
	3	0.014±0.002	0.328±0.029	0.561±0.054
	6	0.014±0.007	0.324±0.038	0.521±0.041
	12	0.016+0.005	0.329+0.011	0.574+0.016
Pine (leached)	Unburied	0.015±0.003	0.401±0.052	0.630±0.046
	3	0.010±0.004	0.330±0.062	0.571±0.033
	6	0.016±0.007	0.337±0.037	0.576±0.018
	12	0.016+0.005	0.349+0.035	0.521+0.032
Spruce	Unburied	0.011±0.004	0.596±0.053	0.905±0.081
	3	0.026±0.009	0.509±0.067	0.869±0.092
	6	0.015±0.007	0.564±0.030	0.888±0.061
	12	0.013+0.003	0.488+0.081	0.896+0.092
Lime	Unburied	0.009±0.002	0.294±0.013	0.439±0.035
	3	0.013±0.002	0.311±0.010	0.484±0.037
	6	0.011±0.005	0.307±0.021	0.475±0.059
	12	0.010+0.002	0.320+0.022	0.442+0.083

Table 3.1.2.13 Chromium contents of wood blocks during the soil burial study (experimental programme 1), determined by analysis. Mean results ± standard deviations are presented (mean is based on a minimum of 5 replicates).

Wood species	Burial time (weeks)	Chromium content (%w/w)		
		Untreated	3%w/v CCA	5%w/v CCA
Pine (unleached)	Unburied	0.003±0.001	0.682±0.054	1.084±0.057
	3	0.005±0.002	0.628±0.052	1.080±0.081
	6	0.005±0.003	0.674±0.043	1.040±0.065
	12	0.009+0.007	0.644+0.046	1.109+0.045
Pine (leached)	Unburied	0.013±0.019	0.786±0.110	1.157±0.047
	3	0.008±0.008	0.650±0.100	1.142±0.076
	6	0.026±0.034	0.666±0.059	1.156±0.044
	12	0.017+0.017	0.690+0.071	1.085+0.101
Spruce	Unburied	0.003±0.002	1.108±0.112	1.699±0.177
	3	0.011±0.001	0.906±0.081	1.696±0.178
	6	0.007±0.004	0.988±0.179	1.696±0.120
	12	0.006+0.002	0.997+0.152	1.696+0.141
Lime	Unburied	0.001±0.001	0.575±0.038	0.961±0.061
	3	0.010±0.011	0.598±0.033	0.897±0.080
	6	0.004±0.002	0.666±0.160	0.923±0.089
	12	0.003+0.001	0.615+0.024	0.920+0.204

Table 3.1.2.14 Arsenic contents wood blocks during the soil burial study (experimental programme 1), determined by analysis. Mean results ± standard deviations are presented (mean is based on a minimum of 5 replicates).

Wood species	Burial time (weeks)	Arsenic content (%w/w)	
		3%w/v CCA	5%w/v CCA
Pine (unleached)	Unburied	0.444±0.057	0.921±0.141
	3	0.509±0.079	0.912±0.052
	6	0.471±0.081	0.859±0.084
	12	0.501+0.058	0.934+0.054
Pine (leached)	Unburied	0.428±0.042	1.032±0.043
	3	0.486±0.099	0.952±0.091
	6	0.471±0.081	0.835±0.166
	12	0.474+0.085	0.909+0.107
Spruce	Unburied	0.881±0.078	1.484±0.254
	3	0.780±0.120	1.305±0.093
	6	0.816±0.152	1.284±0.113
	12	0.786+0.093	1.291+0.077
Lime	Unburied	0.460±0.087	0.746±0.073
	3	0.396±0.042	0.768±0.089
	6	0.376±0.075	0.693±0.168
	12	0.324+0.034	0.669+0.187

Table 3.1.2.15 Results of statistical analyses to investigate possible preservative metal losses from wood blocks during the soil burial study.

Wood species	Metal	One-way analysis of variance		
		Untreated	3%w/v CCA	5%w/v CCA
Pine (unleached)	copper	NS	NS	NS
	chromium	NS	NS	NS
	arsenic	M/N	NS	NS
Pine (leached)	copper	NS	NS	***
	chromium	NS	NS	NS
	arsenic	M/N	NS	*
Spruce	copper	***	*	NS
	chromium	***	NS	NS
	arsenic	M/N	NS	NS
Lime	copper	NS	NS	NS
	chromium	NS	NS	NS
	arsenic	M/N	*	NS

Key M/N Appropriate measurement not carried out.
NS No significant difference.
* Significant difference: probability of difference arising by chance is < 5%.
*** Significant difference: probability of difference arising by chance is < 0.5%.

Table 3.1.2.16 Results of statistical analyses (two-way analysis of variance) to assess differences in preservative metal concentrations of unleached and leached pine blocks with time (experimental programme 1).

Metal	Wood treatment	Interaction	Factor	
			Time	Leaching
Copper	Untreated	NS	NS	NS
	3%w/v CCA	NS	*	NS
	5%w/v CCA	***	NS	*
Chromium	Untreated	NS	NS	NS
	3%w/v CCA	NS	*	NS
	5%w/v CCA	NS	NS	*
Arsenic	Untreated	M/N	M/N	M/N
	3%w/v CCA	NS	NS	NS
	5%w/v CCA	*	*	NS

Key. As table 3.1.2.15.

Table 3.1.2.17 Metal concentrations of soil samples determined by method 1 (see section 2.4.1). Mean results ± standard deviations are presented, along with the estimated percentage recovery of the added metal (mean is based on a minimum of 5 replicates).

Soil type	Metal content (ug g^{-1} soil [dry])			Recovery (%)		
	Copper	Chromium	Arsenic	Cu	Cr	As
Untreated	77.61± 5.98	115.11± 3.21	92.47± 49.26			
+CCA (1)	188.88±14.62	316.33±16.99	180.75±142.95	104.0	105.3	65.6
Untreated	90.44± 5.87	120.17±12.75	M/N			
+CCA (2)	179.77+ 5.24	258.91+41.29	M/N	85.7	74.5	M/N

CCA (1) 106.97ug Cu; 191.04ug Cr; 134.58ug As per gramme soil (dry) estimated to be added.
CCA (2) 104.28ug Cu; 186.23ug Cr estimated to be added.

Table 3.1.2.18 Metal concentrations of soil samples determined by method 2 (i) (see section 2.4.1). Mean results ± standard deviations are presented, along with the estimated percentage recovery of the added metal (mean is based on a minimum of 5 replicates).

Soil type	Metal content (ug g^{-1} soil [dry])			Recovery (%)		
	Copper	Chromium	Arsenic	Cu	Cr	As
Untreated	11.41± 2.33	1.09± 0.32	0.34± 0.56			
+CCA	71.62±10.75	153.33±34.65	2.18± 2.50	55.7	79.0	1.3

+CCA 108.09ug Cu; 192.74ug Cr; 135.74ug As estimated to be added.

Table 3.1.2.17 Metal concentrations of soil samples determined by method 2 (ii) (see section 2.4.1). Mean results ± standard deviations are presented, along with the estimated percentage recovery of the added metal (mean is based on a minimum of 5 replicates).

Soil type	Metal content (ug g^{-1} soil [dry])		Recovery (%)	
	Copper	Chromium	Cu	Cr
Untreated	15.62± 1.87	0.60± 1.11		
+CCA (1)	56.24± 2.59	26.14± 2.28	76.1	26.8
+CCA (2)	96.41± 3.47	62.05± 6.82	75.8	32.3
+CCA (3)	134.07± 5.24	97.63±10.09	76.7	33.2
+CCA (4)	258.02+25.95	214.15+21.78	78.5	36.5

CCA (1) 53.36ug Cu; 95.29ug Cr estimated to be added.
CCA (2) 106.52ug Cu; 190.24ug Cr estimated to be added.
CCA (3) 154.43ug Cu; 292.22ug Cr estimated to be added.
CCA (4) 308.95ug Cu; 584.66ug Cr estimated to be added.

Table 3.1.2.20 Metal concentrations of soil samples determined by method 2 (iii) (see section 2.4.1). Mean results ± standard deviations are presented, along with the estimated percentage recovery of the added metal (mean is based on a minimum of 5 replicates).

Soil type	Metal content (ug g^{-1} soil [dry])		Recovery (%)	
	Copper	Chromium	Cu	Cr
Untreated	20.03± 0.95	1.15± 1.23		
+CCA (1)	66.43± 3.56	34.67± 3.01	87.0	35.2
+CCA (2)	104.66± 4.97	28.00± 2.20	79.4	14.1
+CCA (3)	163.51±12.92	127.88± 7.88	92.9	43.4
+CCA (4)	277.77+16.36	237.27+17.88	83.4	40.4

CCA (1) 53.36ug Cu; 95.29ug Cr estimated to be added.
CCA (2) 106.52ug Cu; 190.24ug Cr estimated to be added.
CCA (3) 154.43ug Cu; 292.22ug Cr estimated to be added.
CCA (4) 308.95ug Cu; 584.66ug Cr estimated to be added.

Table 3.1.2.21 Metal concentrations of soil samples determined by method 3 (see section 2.4.1). Mean results ± standard deviations are presented, along with the estimated percentage recovery of the added metal (mean is based on a minimum of 5 replicates).

Soil type	Metal content (ug g^{-1} soil [dry])			Recovery (%)		
	Copper	Chromium	Arsenic	Cu	Cr	As
Untreated	38.01± 2.62	38.13± 1.56	M/N			
+CCA (1)	47.05± 2.03	57.30± 2.85	M/N	85.2	101.4	M/N
Untreated	36.35± 3.73	22.90± 1.99	M/N			
+CCA (2)	77.58± 5.30	95.46± 6.13	M/N	90.9	95.4	M/N
Untreated	42.39± 1.28	32.79± 1.25	36.11±21.82			
+CCA (3)	116.56+ 4.58	175.26+12.88	96.87+18.50	88.7	94.7	54.2

CCA (1) 10.61ug Cu; 18.90ug Cr estimated to be added.
CCA (2) 45.34ug Cu; 76.04ug Cr estimated to be added.
CCA (3) 83.58ug Cu; 150.44ug Cr; 112.08ug As estimated to be added.

Table 3.1.2.22 Copper contents of soil collected from around buried wood blocks during the soil burial study. Mean results ± standard deviation are presented (mean is based on a minimum of 5 replicates).

Wood species	Burial time (weeks)	Copper content (ug/g soil)		
		Untreated	3%w/v CCA	5%w/v CCA
Pine (unleached)	3	37.0±0.9	59.7±1.8	60.6±5.7
	6	39.1±0.7	60.8±5.9	71.9±58.5
	12	34.9+3.4	75.1+5.4	73.0+20.9
Pine (leached)	3	35.8±3.2	54.2±8.1	48.1±3.7
	6	35.0±2.9	54.8±9.0	58.5±9.9
	12	36.2+6.0	60.6+6.8	65.3+4.1
Spruce	3	40.3±1.7	56.0±9.7	53.2±4.6
	6	39.9±5.3	58.9±9.6	63.0±10.4
	12	38.9+2.9	76.8+20.8	66.4+7.5
Lime	3	43.3±2.5	86.5±12.4	92.0±16.9
	6	42.9±4.1	128.3±60.3	109.7±17.3
	12	42.4+5.4	123.0+18.4	125.5+8.4

Table 3.1.2.23 Chromium contents of soil collected from around buried wood blocks during the soil burial study. Mean results ± standard deviation are presented (mean is based on a minimum of 5 replicates).

Wood species	Burial time (weeks)	Chromium content (ug/g soil)		
		Untreated	3%w/v CCA	5%w/v CCA
Pine (unleached)	3	31.0±8.0	40.0±2.9	36.6±2.6
	6	39.7±0.9	38.8±1.5	38.0±4.8
	12	33.8+1.9	39.1+3.9	36.9+1.2
Pine (leached)	3	30.8±3.4	35.5±3.9	35.1±4.0
	6	33.9±0.9	36.2±4.4	36.6±2.7
	12	35.0+3.4	36.9+1.2	36.4+2.6
Spruce	3	40.6±12.2	41.2±7.4	42.5±7.7
	6	34.7±3.8	34.5±3.3	37.4±4.2
	12	36.1+5.4	37.2+4.4	36.8+4.3
Lime	3	33.3±1.8	50.6±5.7	50.8±12.5
	6	31.6±1.8	48.1±5.0	52.0±6.3
	12	34.5+8.8	55.5+6.0	54.0+3.1

Table 3.1.2.24 Arsenic contents of soil collected from around buried wood blocks during the soil burial study. Mean results ± standard deviation are presented (mean is based on a minimum of 5 replicates).

Wood species	Burial time (weeks)	Arsenic content (ug/g soil)		
		Untreated	3%w/v CCA	5%w/v CCA
Pine (unleached)	3	3.3±6.6	14.3±17.1	23.7±30.1
	6	21.6±18.2	30.6±11.4	33.1±8.7
	12	27.0+16.1	35.8+29.5	33.9+27.4
Pine (leached)	3	17.0±13.0	3.3±4.7	21.4±18.0
	6	21.1±14.8	27.7±19.0	21.2±30.9
	12	13.1+12.9	26.2+24.3	10.9+16.6
Spruce	3	29.8±32.2	32.4±26.4	23.0±19.9
	6	12.2±15.7	43.7±24.0	29.5±51.1
	12	8.0+8.3	42.2+73.1	9.6+16.6
Lime	3	34.8±40.0	57.1±50.4	62.0±39.4
	6	41.5±42.3	149.5±123.6	99.0±89.4
	12	21.5+30.9	272.4+75.5	157.5+37.9

Table 3.1.2.25 Results of statistical analyses (one-way analysis of variance) to investigate changes in preservative metal concentrations (ug g soil) of soil adjacent to wood blocks during the soil burial study.

Wood species	Metal	One-way analysis of variance		
		Untreated	3%w/v CCA	5%w/v CCA
Pine (unleached)	copper	***	***	***
	chromium	NS	**	NS
	arsenic	NS	NS	NS
Pine (leached)	copper	NS	***	***
	chromium	NS	NS	NS
	arsenic	NS	NS	NS
Spruce	copper	NS	***	***
	chromium	NS	NS	NS
	arsenic	NS	NS	NS
Lime	copper	NS	***	***
	chromium	NS	***	***
	arsenic	NS	***	**

Key. NS No significant difference.
* Significant difference: probability of the difference arising by chance is < 5%.
** Significant difference: probability of the difference arising by chance is < 1%.
*** Significant difference: probability of the difference arising by chance is < 0.5%.

Table 3.1.2.26 Results of statistical analyses (two-way analysis of variance) to assess differences in preservative metal concentrations of soil adjacent to untreated, and 3 and 5%w/v CCA-treated wood blocks with time (soil burial 1, experimental programme 1).

Wood species	Metal	Wood treatments	Interaction	Factor	
				Time	Treatment
Pine (unleached)	Copper	0% and 3%	***	***	***
		0% and 5%	***	***	***
		3% and 5%	NS	***	NS
	Chromium	0% and 3%	*	**	*
		0% and 5%	NS	*	NS
		3% and 5%	NS	**	NS
	Arsenic	0% and 3%	NS	NS	NS
		0% and 5%	NS	NS	NS
		3% and 5%	NS	NS	NS
Pine (leached)	Copper	0% and 3%	***	NS	***
		0% and 5%	***	***	***
		3% and 5%	NS	***	NS
	Chromium	0% and 3%	NS	NS	*
		0% and 5%	NS	NS	*
		3% and 5%	NS	NS	NS
	Arsenic	0% and 3%	NS	NS	NS
		0% and 5%	NS	NS	NS
		3% and 5%	NS	NS	NS
Spruce	Copper	0% and 3%	***	***	***
		0% and 5%	***	***	***
		3% and 5%	NS	***	NS
	Chromium	0% and 3%	NS	*	NS
		0% and 5%	NS	*	NS
		3% and 5%	NS	*	NS
	Arsenic	0% and 3%	NS	NS	NS
		0% and 5%	NS	NS	NS
		3% and 5%	NS	NS	NS
Lime	Copper	0% and 3%	***	***	***
		0% and 5%	***	***	***
		3% and 5%	NS	***	NS
	Chromium	0% and 3%	***	***	***
		0% and 5%	***	***	***
		3% and 5%	NS	***	NS
	Arsenic	0% and 3%	***	***	***
		0% and 5%	*	*	*
		3% and 5%	NS	***	NS

Key. 0% Untreated wood.
3% 3%w/v CCA-treated wood.
5% 5%w/v CCA-treated wood.

Remainder of key as table 3.1.2.25

Table 3.1.2.27 Results of statistical analyses (two-way analysis of variance) to assess differences in preservative metal concentrations of soil collected from around unleached and leached pine blocks with time (soil burial 1, experimental programme 1).

Metal	Wood treatment	Interaction	Factor	
			Time	Leaching
Copper	Untreated	NS	*	NS
	3%w/v CCA	NS	***	*
	5%w/v CCA	NS	***	**
Chromium	Untreated	NS	*	NS
	3%w/v CCA	NS	***	*
	5%w/v CCA	NS	*	NS
Arsenic	Untreated	NS	NS	NS
	3%w/v CCA	NS	NS	NS
	5%w/v CCA	NS	NS	NS

Key. As table 3.1.2.25

3.1.3 Soil burial experiment 2.

The effect of pre-burial leaching on the moisture uptake and weight loss of wood blocks during soil burial.

When the moisture contents of the unleached and leached, CCA-treated blocks were compared some results were clearly different, though in other cases differences were not so obvious (figures 3.1.3.2 and 3.1.3.3) Since differences obtained for the unleached and leached, CCA-treated blocks were not always clear, statistical analyses were considered necessary to facilitate comparisons. The variability in groups of moisture content results were very different for the unleached and leached, CCA-treated blocks (table 3.1.3.1); this precluded the use of the usual two-way analysis of variance statistical method. Therefore the Quade test (Conover, 1980) was used (see section 2.6) to determine whether mean moisture contents of unleached and leached wood blocks were different during the study. Weight losses and nitrogen contents were similarly analysed and the results of all statistical analyses are presented in table 3.1.3.5. Due to the relatively limited number of average results produced for each parameter, wood species and preservative treatment, the maximum level of statistical significance which could be achieved was also limited, at $p < 1\%$.

3.1.3.1 Moisture contents of buried wood blocks.

Moisture content results, in the form of mean and standard deviations of each replicate group, are presented in table 3.1.3.1. Average moisture contents are shown in figures 3.1.3.1-3.1.3.3. Note that the scale of the y-axis used in figure 3.1.3.1 is twice that used in the other graphs.

Large standard deviations were obtained for replicate groups of untreated softwood blocks (table 3.1.3.1), though in all cases moisture levels were in excess of the fibre saturation point, and none of the blocks were waterlogged. Since the moisture content of buried wood blocks is related to the moisture content of the soil, it was surmised that the variability in the softwood moisture contents was related to variability of the moisture content of soil used in different boxes. The moisture contents of soil in boxes from which softwood blocks had been sacrificed after 6 weeks of the study could not be measured. However the moisture levels in the remaining softwood boxes were determined to ensure that all soil moisture contents were correct. The moisture content of soil used in this burial experiment was previously determined to be 22.6%w/w at 100% of its water holding capacity (Appendix 2). It was found that, of the remaining 18 boxes containing softwood blocks, 6 had a soil moisture content of approximately 21.3%w/w, while the remaining 12 boxes had a moisture content of about 24.7%w/w. Additional water was added to the former 6 boxes, while the latter 12 boxes were allowed to dry until the moisture content of the soil in all softwood boxes was at approximately 22.6%w/w. The very large standard deviations observed for the moisture contents of untreated softwood blocks were not repeated after the 6 week burial time. All softwood moisture contents from the 6 week time interval have been omitted from graphs and statistical analyses, since there is doubt over their validity.

Similar patterns of moisture uptake were obtained for the unleached and leached, untreated softwood blocks for each wood species (figure 3.1.3.1). No significant differences were found when the data was analysed statistically (table 3.1.3.5). Unleached, untreated lime blocks had significantly greater levels of moisture uptake than did comparable leached blocks (table 3.1.3.5), though

after 12 and 18 weeks of the study these differences were small (figure 3.1.3.1). After 36 weeks of soil burial only about 30% of the original wood substance of the lime blocks remained (table 3.1.3.2), reducing the significance of the moisture content determinations carried out on these blocks.

The average moisture contents of all CCA-treated wood blocks were lower than those of their untreated counterparts (figures 3.1.3.1-3.1.3.3), though they were still in excess of the fibre saturation point.

The moisture contents of all unleached, CCA-treated blocks were consistently greater than those of their leached counterparts (figures 3.1.3.2 and 3.1.3.3); this difference was statistically significant in all cases (table 3.1.3.5). Differences in the moisture contents of the unleached and leached, CCA-treated blocks were not always large, particularly so for the pine and lime blocks (figures 3.1.3.2 and 3.1.3.3).

3.1.3.2 Weight loss of the wood blocks.

Wood weight loss results are presented in table 3.1.3.2 and the average weight loss figures are shown in figures 3.1.3.4-3.1.3.6. Note that the scale of the y-axis in figure 3.1.3.4 is twice that used in figures 3.1.3.5 and 3.1.3.6. The time at which decay of the untreated wood blocks was initiated and the rate of weight loss for each group of blocks were estimated as described in section 3.1.2.2. The results of these estimations are presented in table 3.1.3.3.

All untreated wood blocks exhibited weight losses well in excess of 3% by the conclusion of this study (figure 3.1.3.4). Weight losses of these blocks increased throughout the burial (figure 3.1.3.4). The greatest losses and decay rates were recorded for the hardwood blocks

(figure 3.1.3.4, table 3.1.3.3). Decay also began most rapidly in the buried, untreated lime blocks (table 3.1.3.3). Patterns of weight loss were comparable for unleached and leached, untreated blocks of each wood species (figure 3.1.3.4, table 3.1.3.3). Statistical analyses indicated that leaching had not affected the weight loss results for these blocks (table 3.1.3.5).

Average weight losses of all CCA-treated softwood blocks never exceeded 4%w/w throughout the study (table 3.1.3.2). Average losses of the unleached and leached groups of blocks for both wood species were very similar (figures 3.1.3.5 and 3.1.3.6), with no significant differences being found (table 3.1.3.5).

Average weight losses of unleached and leached, 3%w/v CCA-treated lime blocks were different in the later stages of the experiment (figure 3.1.3.5) and there was a significant difference in the two groups of data (table 3.1.3.5). After 18 weeks of soil burial patches of surface darkening were noted on all 6 replicates of unleached, 3%w/v CCA-treated lime blocks. This darkening was not observed on comparable leached blocks at this time. After 36 weeks of burial brown colouration was again observed on the surface of all replicate unleached, 3%w/v CCA-treated lime blocks and also on 3 of the leached, CCA-treated lime blocks. The standard deviations obtained after 36 weeks of burial were relatively large in both groups (table 3.1.3.2) Leached blocks which had surface discolouration on removal from the soil had weight losses in excess of 4%, while the remaining replicates had not. This accounts for the large standard deviation obtained for this replicate at the final sampling interval.

Average weight losses of the unleached and leached, 5%w/v CCA-treated lime blocks were found to be significantly different (table 3.1.3.5). The graph of these results (figure 3.1.3.6) shows that the average weight losses of the unleached, 5%w/v CCA-treated

lime blocks were consistently greater than those of the leached blocks. However, the difference in weight losses of the unleached and leached blocks was only of the order of 1%w/w. Furthermore, average weight losses of the 5%w/v CCA-treated lime blocks did not increase during the study in either case. This is in contrast with the results of the decaying blocks (untreated blocks of all three wood species and 3%w/v CCA-treated blocks of lime). Thus, it is likely that the 5%w/v CCA-treated lime blocks had not suffered any significant microbial decay. Consequently, the small difference in the weight losses of the unleached and leached, 5%w/v CCA-treated lime blocks is not considered important.

3.1.3.3 Nitrogen contents of 3%w/v CCA-treated lime blocks during a soil burial study.

The nitrogen contents of unleached, 3% CCA-treated lime blocks where decay appeared to have taken place, i.e. after 18 and 36 weeks of soil burial, were determined. For comparison, the nitrogen contents of the corresponding leached blocks were also determined. Average results and standard deviations from these determinations, along with the nitrogen contents of similar unburied, 3%w/v CCA-treated lime blocks (previously presented in section 3.1.2.3), are given in table 3.1.3.4 and figure 3.1.3.7.

After 18 and 36 weeks of soil burial nitrogen contents of both unleached and leached blocks had increased (figure 3.1.3.7). The extent of the difference between nitrogen contents in unleached and leached group of blocks was much greater after soil burial than before burial (figure 3.1.3.7). Furthermore over the entire study the nitrogen contents of the unleached blocks were significantly greater than those of the leached blocks (table 3.1.3.4). Relatively large

standard deviations were recorded for the nitrogen contents of both sets of blocks after 36 weeks, particularly in the leached group (table 3.1.3.4). In the case of the leached blocks, 4 replicates at the 36 week burial time had nitrogen contents of less than 0.15%w/w, while the remaining 2 had contents in excess of 0.2%w/w, the latter being 2 of the 3 blocks which had surface darkening on removal from soil and had weight losses in excess of 4%w/w.

3.1.3.4 Figures 3.1.3.1-3.1.3.7

Figure 3.1.3.1 Average moisture contents of untreated blocks during the soil burial study.

Figure 3.1.3.2 Average moisture contents of 3%w/v CCA-treated blocks during the soil burial study.

Figure 3.1.3.3 Average moisture contents of 5%w/v CCA-treated blocks during the soil burial study.

Figure 3.1.3.4 Average weight losses of untreated blocks during the soil burial study.

Figure 3.1.3.5 Average weight losses of 3%w/v CCA-treated blocks during the soil burial study.

Figure 3.1.3.6 Average weight losses of 5%w/v CCA-treated blocks during the soil burial study.

Figure 3.1.3.7 Average nitrogen contents of 3%w/v CCA-treated lime blocks during the soil burial study.

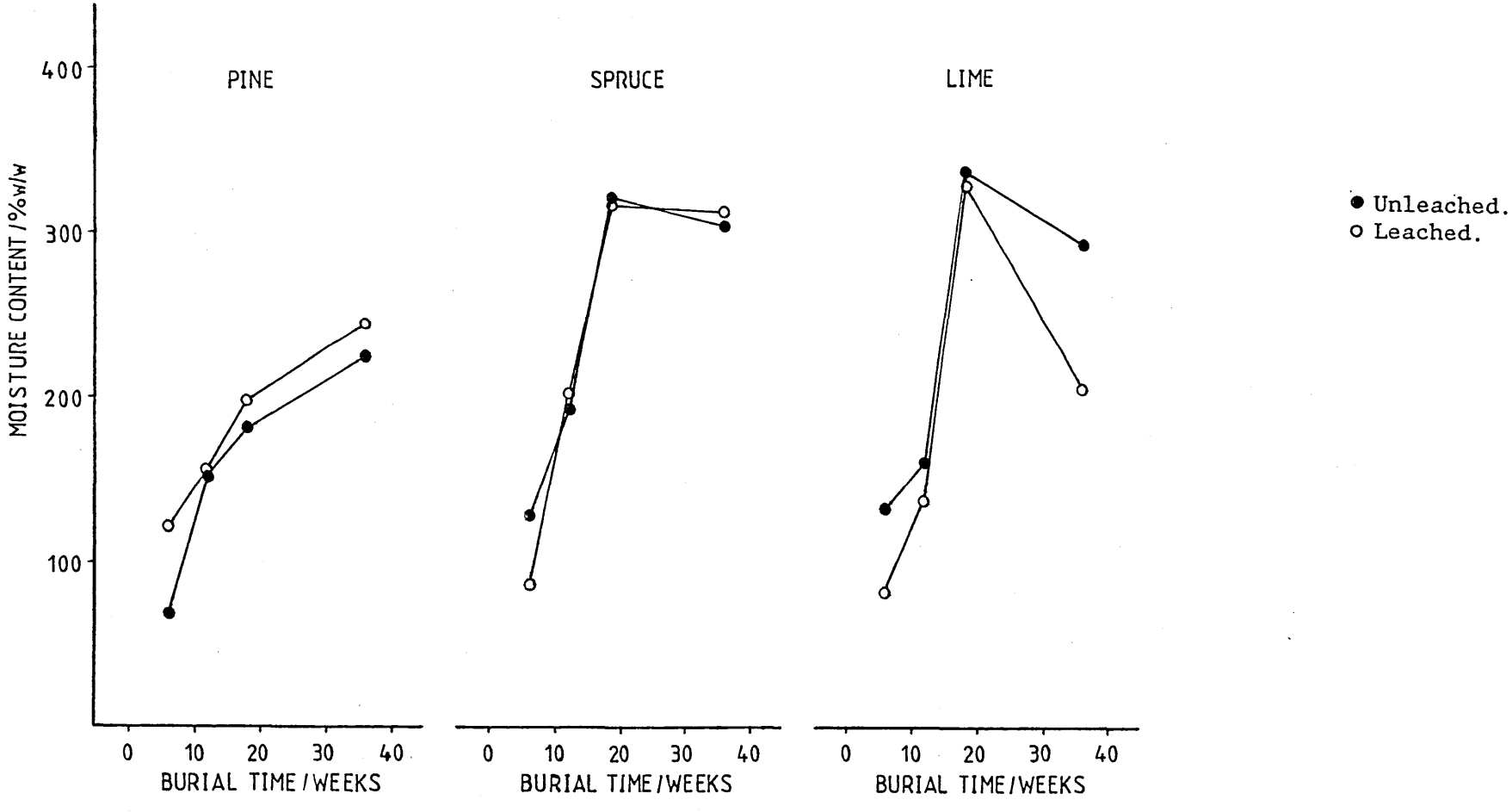


Figure 3.1.3.1 Average moisture contents of untreated blocks during the soil burial study. Average is based on a minimum of 5 replicates.

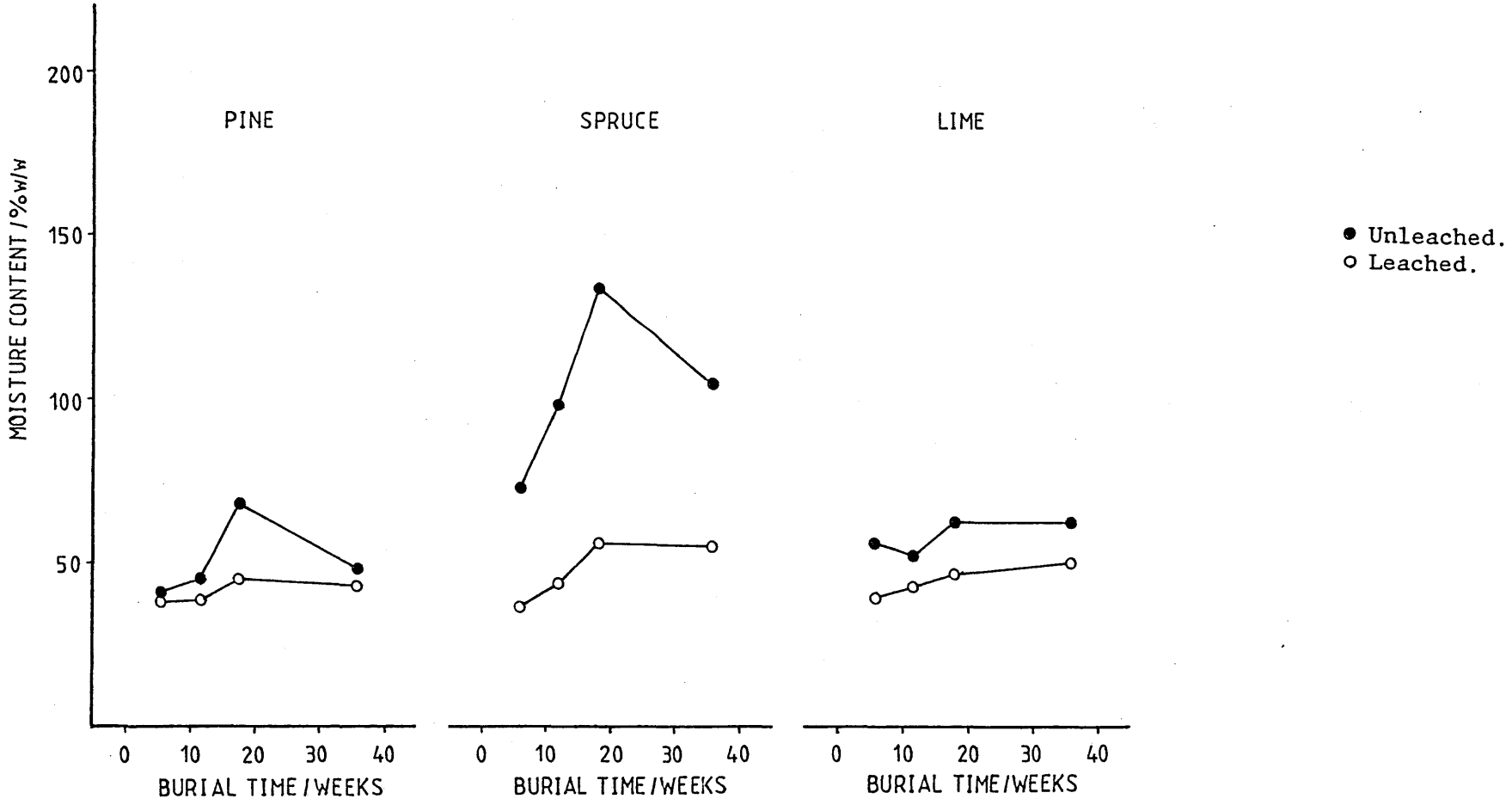


Figure 3.1.3.2 Average moisture contents of 3%w/v CCA-treated blocks during the soil burial study. Average is based on a minimum of 5 replicates.

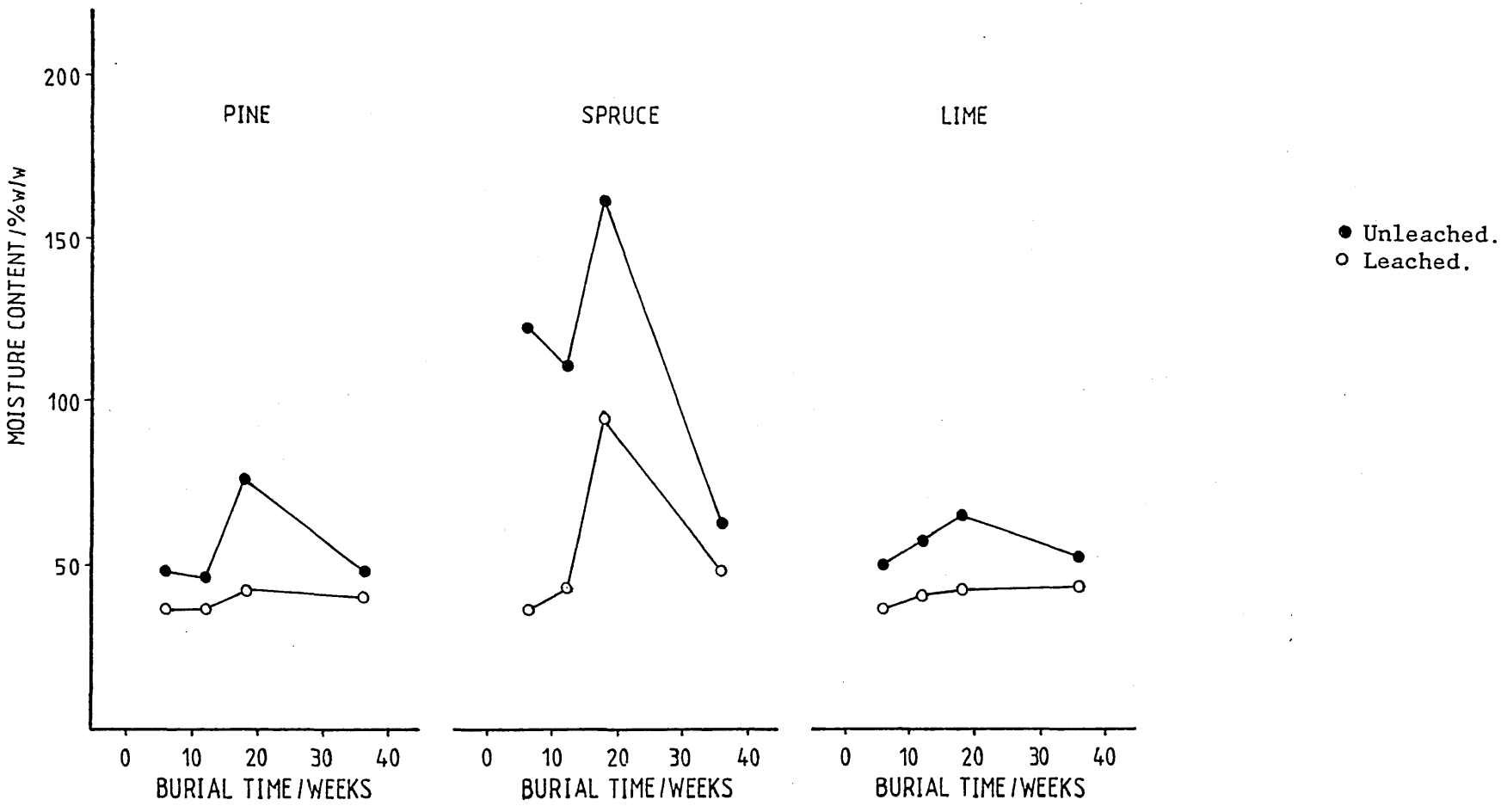


Figure 3.1.3.3 Average moisture contents of 5%w/v CCA-treated blocks during the soil burial study. Average is based on a minimum of 5 replicates.

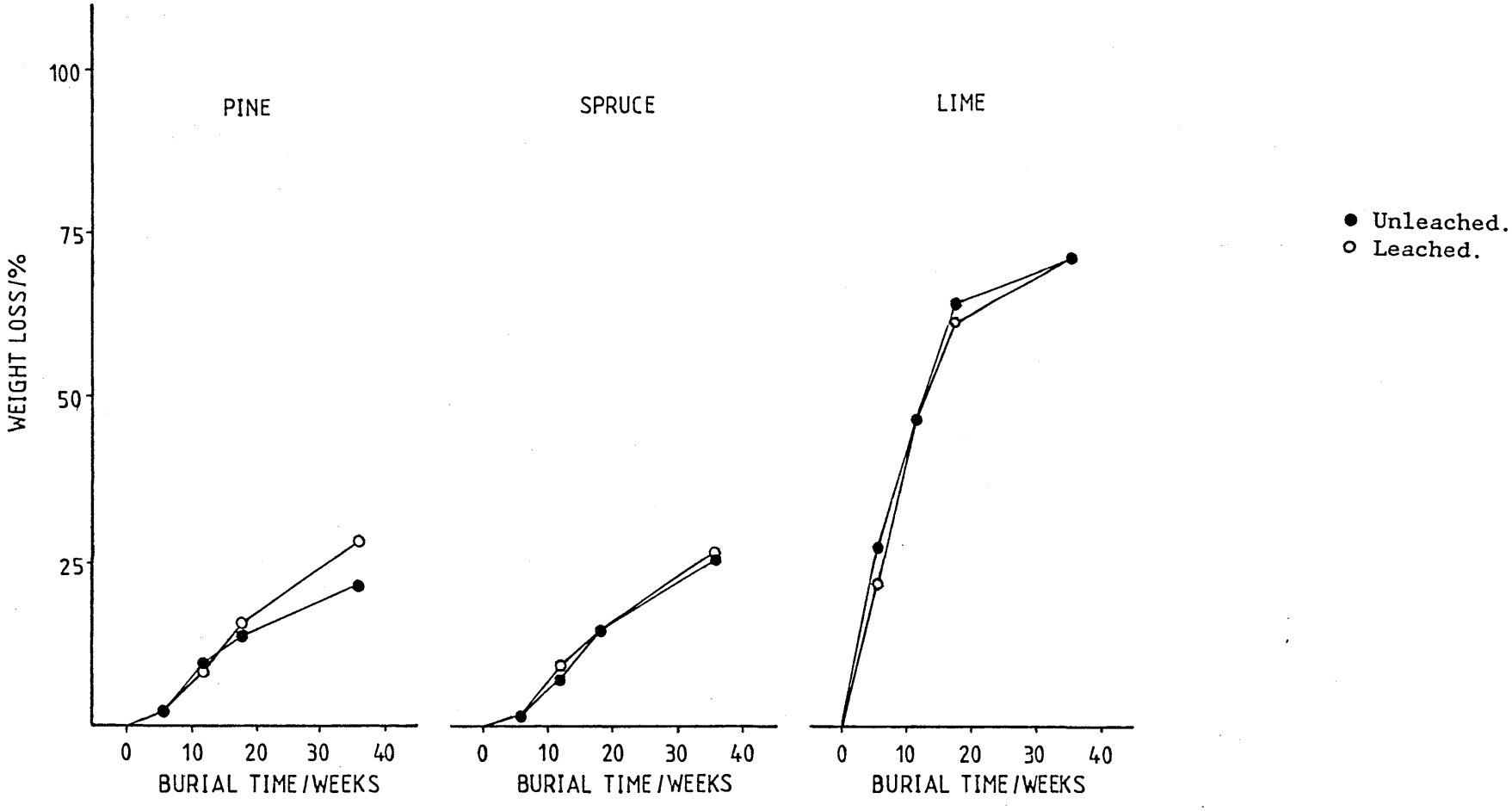


Figure 3.1.3.4 Average weight losses of untreated blocks during the soil burial study. Average is based on a minimum of 5 replicates.

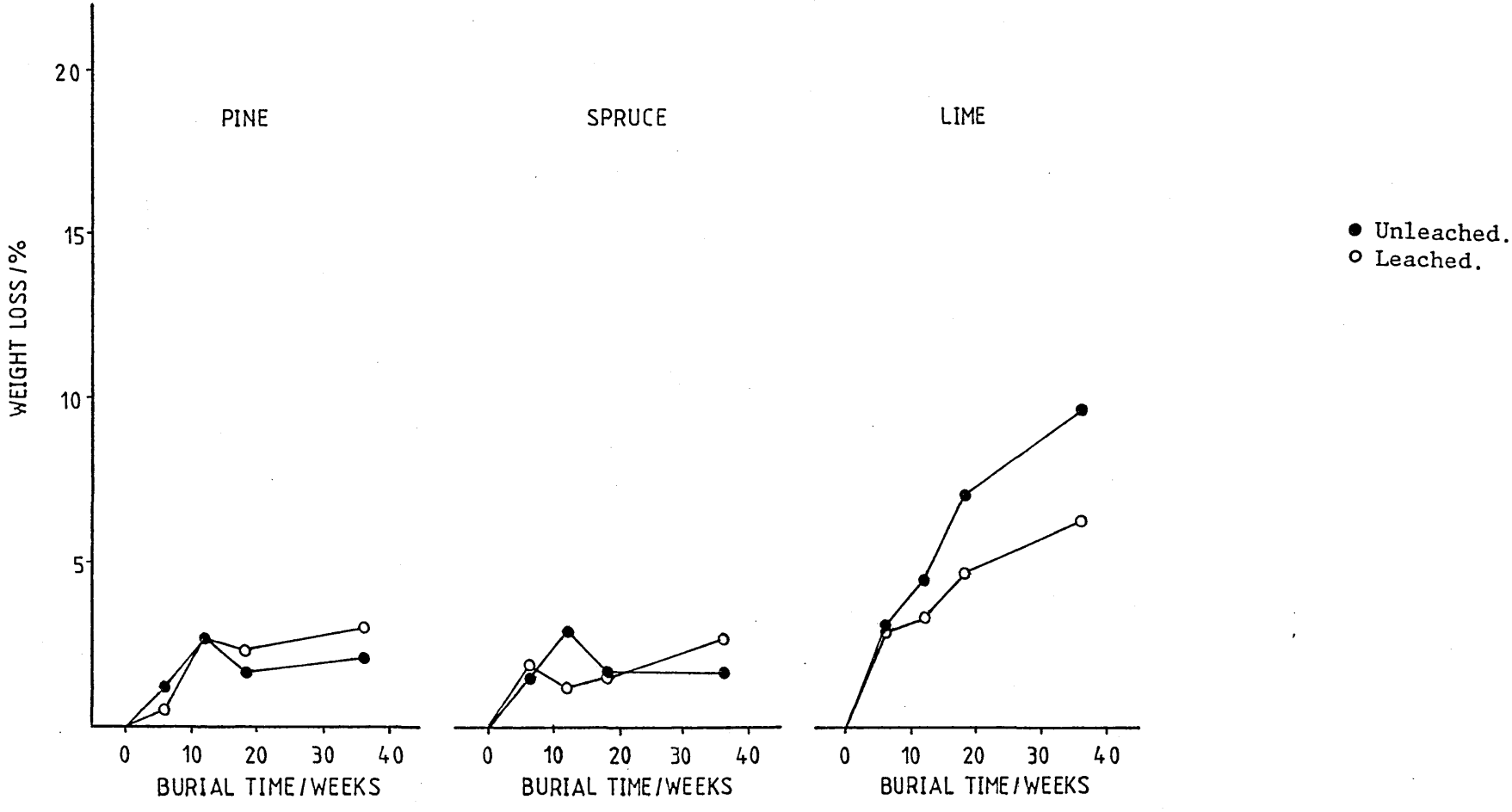


Figure 3.1.3.5 Average weight losses of 3%w/v CCA-treated blocks during the soil burial study. Average is based on a minimum of 5 replicates.

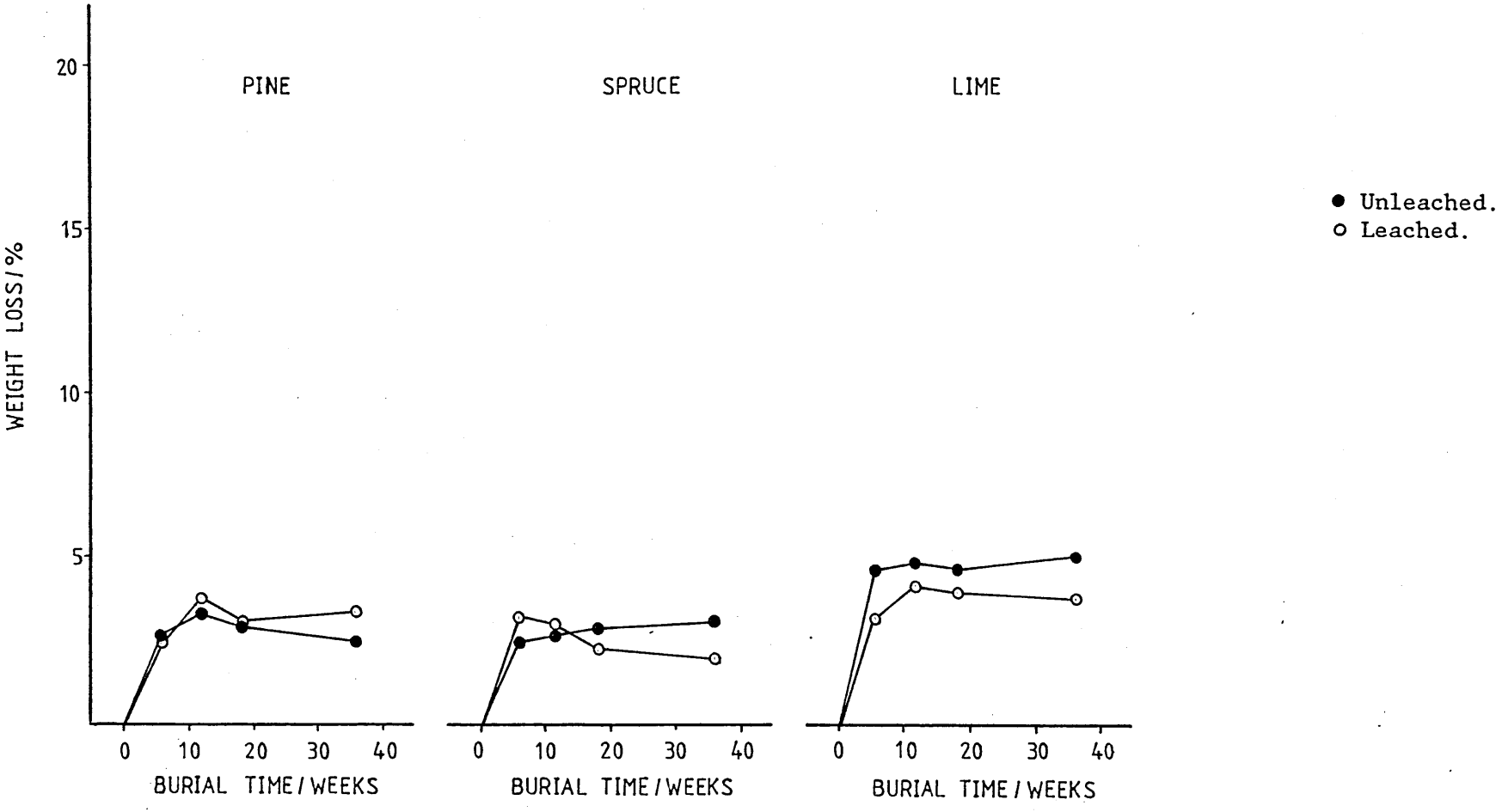


Figure 3.1.3.6 Average weight losses of 5%w/v CCA-treated blocks during the soil burial study. Average is based on a minimum of 5 replicates.

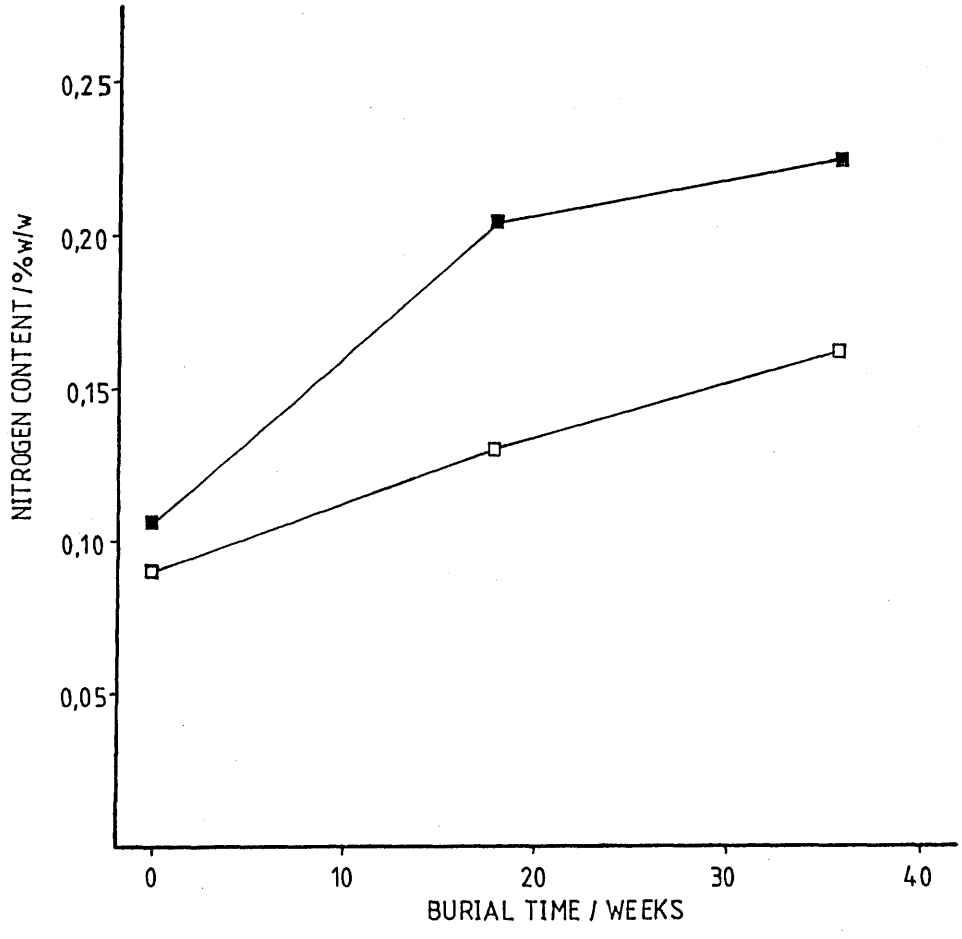


Figure 3.1.3.7 Average nitrogen contents of 3%w/v CCA-treated lime blocks during the soil burial study. Average is based on a minimum of 5 replicates.

Unleached. ■
Leached. □

3.1.3.5 Tables 3.1.3.1-3.1.3.5.

Table 3.1.3.1 Moisture contents of wood blocks during soil burial study 2, experimental programme 1.

Table 3.1.3.2 Weight losses of wood blocks during soil burial study 2, experimental programme 1.

Table 3.1.3.3 Estimated time to significant microbial decay of untreated wood blocks and subsequent rate of decay for each wood species during the study.

Table 3.1.3.4 Nitrogen contents of 3%w/v CCA-treated lime blocks during soil burial study 2, experimental programme 1.

Table 3.1.3.5 Statistical comparison of the average moisture contents, weight losses and nitrogen contents of unleached and leached blocks after soil burial.

Table 3.1.3.1 Moisture contents of wood blocks during soil burial study 2, experimental programme 1. Mean results ± standard deviations are presented (mean is based on a minimum of 5 replicates).

Wood species	Burial time (weeks)	Leaching	Moisture content (%w/w)		
			Untreated	3%w/v CCA	5%w/v CCA
Pine	6	Not leached	69.3±46.4	40.9±4.3	47.4±8.2
		Leached	119.6±51.2	37.2±2.1	36.2±2.2
	12	Not leached	153.6±15.7	45.0±2.9	45.8±2.7
		Leached	152.0±33.4	38.0±1.0	36.3±0.3
	18	Not leached	179.6±30.7	68.1±10.5	76.2±17.4
		Leached	197.6±23.5	45.1±3.4	42.2±2.8
	36	Not leached	225.1±22.2	48.7±8.4	48.2±5.3
		Leached	245.0+30.8	42.6+1.4	39.5+1.6
Spruce	6	Not leached	126.7±105.8	71.6±16.8	122.9±29.1
		Leached	86.3±75.9	36.5±3.5	35.8±3.4
	12	Not leached	190.8±47.4	97.9±17.4	110.1±15.8
		Leached	201.5±55.6	43.1±10.7	44.9±9.2
	18	Not leached	321.8±18.7	133.7±28.8	160.9±33.9
		Leached	317.2±32.3	55.7±19.1	93.8±9.5
	36	Not leached	302.4±70.7	104.5±48.5	63.0±10.0
		Leached	312.9+39.1	54.8+19.2	47.7+4.4
Lime	6	Not leached	132.0±28.8	56.9±2.0	49.6±1.8
		Leached	81.1±27.8	39.0±1.1	36.9±1.1
	12	Not leached	161.1±56.0	52.6±3.7	57.0±2.6
		Leached	137.8±52.5	41.6±1.3	39.4±1.2
	18	Not leached	337.8±67.8	61.6±13.2	64.8±2.6
		Leached	328.8±79.6	45.8±1.7	42.0±1.2
	36	Not leached	290.7±42.1	62.2±8.9	52.1±4.6
		Leached	205.2+31.8	49.7+7.6	41.9+1.7

Table 3.1.3.2 Weight losses of wood blocks during soil burial study 2, experimental programme 1. Mean results ± standard deviations are presented (mean is based on a minimum of 5 replicates).

Wood species	Burial time (weeks)	Leaching	Weight loss (%)		
			Untreated	3%w/v CCA	5%w/v CCA
Pine	6	Not leached	1.63±2.11	1.10±2.52	2.64±1.81
		Leached	2.07±0.49	0.34±3.44	2.53±0.40
	12	Not leached	8.69±1.75	2.55±0.40	3.18±0.19
		Leached	7.92±1.86	2.70±0.16	3.72±0.44
	18	Not leached	13.20±3.69	1.61±0.19	2.75±0.37
		Leached	14.59±2.91	2.21±0.25	2.96±0.23
	36	Not leached	20.93±4.17	2.04±0.27	2.61±0.76
		Leached	27.76+6.10	2.89+0.32	3.37+0.45
Spruce	6	Not leached	0.80±1.24	1.44±2.93	2.54±5.20
		Leached	0.93±0.89	1.79±0.52	3.14±0.59
	12	Not leached	6.47±1.65	2.74±1.74	2.66±1.02
		Leached	8.56±1.70	1.08±1.82	2.94±0.44
	18	Not leached	14.03±1.28	1.71±0.35	2.85±0.94
		Leached	13.89±0.90	1.41±0.62	2.27±0.41
	36	Not leached	25.35±1.83	1.64±0.47	2.96±0.93
		Leached	26.38+3.07	2.59+0.37	2.07+0.76
Lime	6	Not leached	26.67±7.01	3.04±0.21	4.63±0.62
		Leached	21.02±9.64	2.72±0.47	3.32±0.54
	12	Not leached	46.10±12.54	4.49±3.07	4.77±2.46
		Leached	46.23±9.79	3.29±0.34	4.17±0.17
	18	Not leached	64.54±5.36	7.03±1.80	4.65±0.63
		Leached	62.47±7.56	4.70±0.44	4.01±0.49
	36	Not leached	70.56±3.97	9.64±4.74	4.95±0.97
		Leached	71.37+1.75	6.25+4.35	3.82+0.57

Table 3.1.3.3 Estimated time to significant microbial decay of untreated wood blocks and subsequent rate of decay for each wood species during the study.

Wood species	Leaching	Initiation of decay (weeks)	Rate of decay (%/week)
Pine	Not leached	7.10	0.62
	Leached	6.75	0.85
Spruce	Not leached	8.30	0.81
	Leached	7.50	0.82
Lime	Not leached	0.70	1.91
	Leached	0.70	1.94

Table 3.1.3.4 Nitrogen contents of 3%w/v CCA-treated lime blocks during soil burial study 2, experimental programme 1. Means and standard deviations are presented (mean is based on a minimum of 5 replicates).

Burial time (weeks)	Nitrogen content(%w/w).	
	Unleached blocks	Leached blocks
Unburied	0.106±0.019	0.091±0.017
18	0.203±0.028	0.131±0.017
36	0.226±0.043	0.162±0.057

Table 3.1.3.5 Statistical comparison of the average moisture contents, weight losses and nitrogen contents of unleached and leached wood blocks after soil burial.

Wood species	Preservative concentration (%w/w)	Moisture content (%w/w)	Weight loss (%)	Nitrogen content (%w/w)
Pine	Untreated	NSD	NSD	N/D
	3	***	NSD	N/D
	5	***	NSD	N/D
Spruce	Untreated	NSD	NSD	N/D
	3	***	NSD	N/D
	5	***	NSD	N/D
Lime	Untreated	***	NSD	N/D
	3	***	***	N/D
	5	***	***	***

Key. NSD No significant difference.
*** Significant difference: probability of the difference arising by chance is < 1%.
N/D Comparison not carried out.

3.2 Experimental programme 2.

The effect of CCA on microbial activity in the wood-soil system.

The standard deviations obtained for dehydrogenase activities in replicate groups of wood and soil samples were often very large and on occasions in excess of the average results (tables 3.2.12-3.2.17). Therefore, individual assay results are presented on the relevant graphs. A curve is drawn through the average values of each set of 4 replicate assays (figures 3.2.3-3.2.9).

One-way analysis of variance was carried out on all wood nitrogen contents (see section 2.6); the results are given in tables 3.2.6 and 3.2.8.

A replicate group of untreated lime blocks were exhumed after 2 weeks of soil burial and their moisture contents, weight losses and nitrogen contents measured. The results are presented in the appropriate tables and figures. However, dehydrogenase activity results were not obtained for these wood blocks or for their adjacent soil, due to technical difficulties.

3.2.1 Moisture contents of buried wood blocks.

Average moisture contents and standard deviations of the softwood blocks and lime blocks in this study are presented in tables 3.2.1 and 3.2.2 respectively.

The moisture contents of all wood blocks uplifted during this study were in excess of the fibre saturation point (tables 3.2.1 and 3.2.2). Moisture contents of the untreated wood blocks were greater than those of the CCA-treated blocks for each wood species. However, increasing the preservative concentration in the wood blocks did not reduce the amount of moisture taken up from the soil. Instead the

5%w/v CCA-treated spruce blocks always had substantially greater moisture contents than the 0.25%w/v CCA-treated spruce blocks (table 3.2.1).

There was a substantial increase in the moisture contents of all untreated wood blocks and 0.5%w/v CCA-treated lime blocks with time (tables 3.2.1 and 3.2.2); the levels in the 0.25%w/v CCA-treated pine also increased during the study. The moisture contents in the remaining CCA-treated wood blocks remained either constant, or decreased slightly during the study (tables 3.2.1 and 3.2.2).

3.2.2 Weight loss of the wood blocks.

Average weight loss results and standard deviations are given in tables 3.2.3 (softwoods) and 3.2.4 (lime), and the average values are also shown in figure 3.2.1. The time at which decay of the blocks was initiated and the subsequent rate of decay were estimated by the method described in section 3.1.2.2; the results are given in tables 3.2.5 and 3.2.6 respectively.

Weight losses of all untreated blocks were greater than 3% by the conclusion of this 24 week study (figure 3.2.1). Significant weight loss of the untreated lime blocks was measured within a few weeks of setting up this experiment (table 3.2.5). The most rapid rate of weight loss was also measured for the untreated hardwood blocks (table 3.2.6). Untreated pine and spruce weight loss results were similar (figure 3.2.1), though decay began more rapidly in the spruce blocks (table 3.2.5) and proceeded at a slightly faster rate (table 3.2.6).

Decay of the 0.5%w/v CCA-treated lime blocks and untreated lime blocks began soon after their emplacement in soil (table 3.2.5). The rate of weight loss of the 0.5%w/v CCA-treated lime blocks was

greater than observed for either of the untreated softwood blocks, but less than the rate for the untreated lime blocks (figure 3.2.1; table 3.2.6).

Pine blocks treated with a 0.25%w/v CCA solution had an average weight loss of 4.3% by the conclusion of this experiment, though before this, average weight loss never exceeded 2% (figure 3.2.1). Thus, microbial decay of these blocks was considered to have begun after about 22 weeks of soil burial (table 3.2.5). Their rate of weight loss was the same as for the untreated pine blocks (table 3.2.6), though the short time range over which this rate was determined limits its accuracy.

Average weight loss values for the 0.25%w/v CCA-treated spruce blocks and the 5%w/v CCA-treated pine blocks never exceeded 3% in this study (figure 3.2.1). At the first sampling interval average weight loss of the 5%w/v CCA-treated spruce and lime blocks was in excess of 3%. However, since the level of weight loss did not increase significantly during the study, and since the 0.25%w/v CCA-treated spruce blocks did not fail (figure 3.2.1) it is considered that the 5%w/v CCA-treated spruce and lime blocks were not decaying.

3.2.3 Wood block nitrogen contents.

Average nitrogen contents of all replicate groups of wood blocks are shown in figure 3.2.2 and tables 3.2.7 (softwoods) and 3.2.9 (lime). Standard deviations are also presented in these tables. Results of statistical analyses are given in tables 3.2.8 (softwoods) and 3.2.10 (lime). The nitrogen contents of the wood blocks at the time that soft rot decay was initiated were determined as described in section 3.1.2.3; the results are presented in table 3.2.11.

The nitrogen contents of the 0.5%w/v CCA-treated lime blocks and the untreated blocks of all wood species increased throughout the study (figure 3.2.2). These increases were determined to be significant ($p < 0.5\%$, tables 3.2.8 and 3.2.10). Figure 3.2.2 shows the rate of nitrogen increase in the 0.5%w/v CCA-treated lime blocks was less than the rate for the untreated lime blocks. The nitrogen contents of the 0.25%w/v CCA-treated pine blocks increased slightly during the study (figure 3.2.2); this increase was highly significant ($p < 0.5\%$, table 3.2.8).

Blocks of 0.5%w/v CCA-treated lime, 0.25%w/v CCA-treated pine and untreated blocks of all three wood species appeared to suffer some microbial decay during this study (see section 3.2.2). At the time microbial decay was initiated the nitrogen contents of all these blocks were greater than those for the equivalent unburied blocks (tables 3.2.7, 3.2.9, 3.2.11).

A small, significant ($p < 5\%$) increase in the average nitrogen contents of the 0.25%w/v CCA-treated spruce blocks occurred after 6 weeks of soil burial, though no further increases were measured (figure 3.2.2, table 3.2.8). Nitrogen contents of 5%w/v CCA-treated softwood blocks did not increase during the first 12 weeks of the experiment, though at the conclusion of this study a small, significant ($p < 5\%$) increase in their nitrogen contents was evident (figure 3.2.2, table 3.2.8). The nitrogen contents of similar lime blocks had also increased slightly by the end of this experiment (figure 3.2.2), though statistical analysis indicated that this increase was not significant (table 3.2.10).

3.2.4 Levels of dehydrogenase activity in the outer wood surface of buried wood blocks.

Levels of dehydrogenase activity measured in the outer wood surface of wood blocks during this soil burial study are shown in figures 3.2.3 (softwoods) and 3.2.4 (lime) and in tables 3.2.12 (softwoods) and 3.2.13 (lime).

Dehydrogenase activity was always detected in the outer wood surface of the untreated blocks of all three wood species (figures 3.2.3 and 3.2.4). Average activity levels in the outer wood surface of untreated pine blocks increased throughout the 24 weeks of the study (figure 3.2.3). However, the greatest activity level in any untreated pine block was recorded after 12 weeks of the experiment. Generally activity values measured in the untreated pine blocks exhibited limited variability (figure 3.2.3, table 3.2.12).

Dehydrogenase activities in untreated spruce blocks were more variable and generally lower than activities in the untreated pine blocks (figure 3.2.3). Average activities in the untreated spruce blocks did not increase throughout the study, but rather peaked at the 9 week sampling interval.

The greatest activity levels were measured in the untreated lime blocks (figure 3.2.4). The maximum average level and greatest variation of dehydrogenase activity in the untreated lime blocks was measured after 4 weeks of the study (figure 3.2.4, tables 3.2.12 and 3.2.13). After 6 and 12 weeks the levels of activity in the untreated lime blocks were similar to those measured in the untreated pine blocks in the latter half of the study (figures 3.2.3. and 3.2.4).

Dehydrogenase activity was measured in all 0.5%w/v CCA-treated lime blocks during this study. Lower levels of activity were measured in the 0.5%w/v CCA-treated lime blocks than in untreated blocks of

all three wood species (figure 3.2.4). Average activities increased during the first 9 weeks of the soil burial study, then remained approximately constant during the remainder of the burial. Dehydrogenase activities in the outer surface of 0.5%w/v CCA-treated lime blocks were generally less variable than those in the untreated blocks of all three wood species (figures 3.2.3 and 3.2.4, tables 3.2.12 and 3.2.13).

Very low activity levels were occasionally measured in the 0.25%w/v CCA-treated pine blocks (table 3.2.12). No dehydrogenase activity was detected in the outer wood surface of 0.25%w/v CCA-treated spruce blocks and 5%w/v CCA-treated blocks of all three wood species (tables 3.2.12 and 3.2.13).

3.2.5 Levels of dehydrogenase activity in the inner wood of buried wood blocks.

Dehydrogenase activity levels in the inner wood of wood blocks are presented in tables 3.2.14 (softwoods) and 3.2.15 (lime). Results are also shown in figures 3.2.5 (softwoods) and 3.2.6 (lime).

Activity was measured in the inner wood of all untreated pine blocks in the soil burial (figure 3.2.5). However, the levels were always lower than those of the corresponding outer wood surfaces (figure 3.2.3). Levels of dehydrogenase activity in the inner wood of the untreated pine blocks generally increased during the study (figure 3.2.5).

Dehydrogenase activity in the inner wood of untreated spruce blocks reached a maximum after 9 weeks (figure 3.2.5). Dehydrogenase levels in the inner wood of the untreated spruce blocks were also consistently lower than those of the corresponding outer wood

surfaces (figures 3.2.5 and 3.2.3). By the conclusion of the study levels of activity in the untreated spruce blocks were far lower than those in the inner wood of the untreated pine and lime blocks.

Dehydrogenase activity was always measured in the inner wood of untreated lime blocks. The greatest average level was measured after 4 weeks of the study (figure 3.2.6), as was found for the outer surface of these blocks (figure 3.2.4). A very large standard deviation of ± 215 was obtained for the untreated lime blocks uplifted after 4 weeks of soil burial (table 3.2.15). This was due to a single result of $473*10^{-5}$ umol g^{-1} min^{-1} , which was far greater than any of the readings obtained for the other replicate blocks (figure 3.2.6). This value was also in excess of the dehydrogenase activity level in the corresponding outer surface of this wood block. This was the only occasion when the level of dehydrogenase activity in the inner wood was greater than that in the outer wood surface of the same block.

Very low levels of activity were measured in the inner wood of all 0.5%w/v CCA-treated lime blocks (figure 3.2.6). Levels of dehydrogenase activity in these blocks generally increased throughout the study (figure 3.2.6). At the 24 week sampling time activity in the 0.5%w/v CCA-treated lime blocks was less than in the untreated lime and pine blocks, but greater than in the untreated spruce blocks (figures 3.2.5 and 3.2.6).

Samples of the inner wood of CCA-treated softwood blocks and 5%w/v CCA-treated lime blocks were assayed for dehydrogenase activity; no activity was detected.

3.2.6 Levels of dehydrogenase activity in soil adjacent to buried wood blocks and in soil at a distance from these blocks.

The dehydrogenase activity in soil adjacent to wood blocks are presented in tables 3.2.16 (softwoods) and 3.2.17 (lime) and in figures 3.2.7-3.2.9. Soil samples collected at a distance from any buried wood blocks (controls) were also assayed for dehydrogenase activity levels. A total of four replicate samples were collected from boxes containing soil at 80% of its water holding capacity and a further four from boxes which contained soil at 100% of its water holding capacity. Activity levels in the latter group of soil samples are reported with both the pine and the spruce results. All average activity values are shown in figure 3.2.10.

Four replicate soil samples were assayed for dehydrogenase activity immediately before setting up this soil burial study; the level of dehydrogenase activity was found to be $0.9 \pm 0.6*10^{-5}$ umol g^{-1} min^{-1} . This level of activity represents the zero time level in all figures presented in this section.

Dehydrogenase activities in hardwood and softwood control samples had increased by the first sampling interval (figures 3.2.7-3.2.10). Subsequently activity in these samples fell and remained near the zero time level for the rest of the experiment. Dehydrogenase activity had increased in the softwood controls by the 18 week sampling time, although 6 weeks later the activity returned to former levels.

After 3 weeks the average dehydrogenase activity had increased in soil adjacent to all pine blocks (figures 3.2.7 and 3.2.10). At the 3 week sampling time the greatest level of activity around any buried pine blocks was measured in soil adjacent to the untreated blocks.

Levels of activity in soil around 0.25 and 5%w/v CCA-treated pine blocks were similar at this time and these values were greater than those of the controls (figures 3.2.7 and 3.2.10). Activity in soil adjacent to untreated pine blocks fell slightly at the 6 and 9 week burial times, though it was always greater than levels in the corresponding control samples (figures 3.2.7 and 3.2.10). Subsequently the average activity in soil adjacent to untreated pine blocks increased again, as did the variability (table 3.2.16, figure 3.2.7). Dehydrogenase activity in soil adjacent to untreated pine blocks was greater than in either the soil from around CCA-treated pine blocks or the control samples (figures 3.2.7 and 3.2.10).

The initial increase in activity in soil adjacent to 0.25%w/v CCA-treated pine blocks was not sustained; after a further 3 weeks activity around these blocks had fallen (figures 3.2.7 and 3.2.10). At the 6 week sampling time dehydrogenase activity around the 0.25%w/v CCA-treated pine blocks was greater than activities in soil adjacent to the 5%w/v CCA-treated pine blocks and in the controls (figures 3.2.7 and 3.2.10). After 12 weeks the activity in soil adjacent to the 0.25%w/v CCA-treated pine blocks was at the same level as the control samples. Later the activity around these blocks increased to levels slightly greater than control levels (figures 3.2.7 and 3.2.10).

The greatest levels of activity measured in soil adjacent to 5%w/v CCA-treated pine blocks were obtained after 3 weeks (figures 3.2.7 and 3.2.10). By week 6 activity had fallen significantly and was approaching the control level. Activities in samples from around 5%w/v CCA-treated pine blocks were similar to control values for the remainder of the experiment (figures 3.2.7 and 3.2.10).

By the first sampling interval dehydrogenase activities in soil adjacent to all spruce blocks had increased (figures 3.2.8 and

3.2.10). The average level of activity in soil adjacent to untreated spruce blocks after 3 weeks was the greatest recorded for soil adjacent to any group of spruce blocks during this experiment. After a further 3 weeks activities in soil adjacent to untreated spruce blocks had fallen, though they remained significantly greater than those of any other spruce samples. A small increase was observed in subsequent weeks, though the levels were lower than those in soil adjacent to untreated pine blocks. The average levels of activity in soil adjacent to untreated spruce blocks were always greater than either the control samples, or those from around CCA-treated spruce blocks (figure 3.2.10).

After the 3 week sampling time activity in soil around the 0.25%w/v CCA-treated spruce blocks fell during the subsequent 9 weeks and approached the control level (figures 3.2.8 and 3.2.10). However, at the 18 week burial time increased levels of dehydrogenase activity were measured in soil around these blocks, though activity in the control samples was also increased at this time. By the conclusion of the experiment activities in soil collected from around the 0.25%w/v CCA-treated spruce blocks had again fallen and were similar to those of the control samples.

Activity in soil adjacent to 5%w/v CCA-treated spruce blocks peaked after 3 weeks of soil burial (figures 3.2.8 and 3.2.10). However, the levels measured at this time were only slightly greater than the control levels. The activity in soil adjacent to 5%w/v CCA-treated spruce blocks fell during the subsequent weeks. By the 12 week sampling interval the average activity was less than the control level. At this sampling time no dehydrogenase activity was detected in three of the four replicate samples from around the treated spruce blocks. This was also the case for two replicate samples at the 24 week burial time. These were the only occasions on which no activity

was measured in soil samples.

The greatest soil dehydrogenase activity measurements in this study were obtained for soil adjacent to untreated lime block after 3 weeks (figures 3.2.9 and 3.2.10). The average level of activity in soil adjacent to these blocks had fallen by the 4 week burial time and continued to decrease during the remainder of the experiment. Despite this decrease in activity, average dehydrogenase levels in soil adjacent to untreated lime blocks exceeded those of any other replicate group of soil samples (figure 3.2.10).

Similar average levels of dehydrogenase activity were measured in soil adjacent to 0.5 and 5%w/v CCA-treated lime blocks at the 3 week burial time (figures 3.2.9 and 3.2.10). Microbial activity measured around these blocks after 3 weeks was greater than the activity levels in any other replicate group of samples, with the exception of the untreated lime blocks. The increase in dehydrogenase activity in soil surrounding the 0.5%w/v CCA-treated lime blocks was sustained for a further 3 weeks. By the ninth week activity around these blocks had fallen significantly, though the average level was still greater than those in the softwood soil samples (figure 3.2.10). Dehydrogenase activity around the 0.5%w/v CCA-treated lime blocks decreased slightly during the remainder of this study.

Following the peak of activity at 3 weeks, the activity in soil adjacent to 5%w/v CCA-treated lime blocks decreased rapidly (figures 3.2.9 and 3.2.10). However, it was not until the twelfth week that the dehydrogenase levels around these treated blocks approached the control soil levels. During the remainder of the soil burial study levels of microbial activity measured in soil around the 5%w/v CCA-treated lime blocks were similar to the control levels.

3.2.7 Experimental check on the dehydrogenase method.

The average recoveries of added TTF from soil and untreated wood samples were between 85 and 93% (table 3.2.18), irrespective of wood species, rinsing technique and the amount of TTF added. Substantially reduced recoveries of TTF were measured from CCA and ACA-treated wood samples (table 3.2.18). Recovery appeared to be partly dependent on the amount of salt within the wood, since samples of 0.07 and 1.41%w/v ACA-treated spruce to which the same amount of TTF had been added, had recoveries of 38.6 and 22.0% respectively (table 3.2.18). However, the amount of TTF recovered was dependent on the quantity added to the samples. Sufficient TTF was added to 3%w/v CCA-treated lime samples to give an average absorbance of 1.019; 62.0% of this was recovered. The control absorbance for the 5%w/v CCA-treated spruce samples was 0.088; 26.7% of this was recovered (table 3.2.18).

3.2.8 Figures 3.2.1-3.2.10.

Figure 3.2.1 Average weight losses of wood blocks during the soil burial study.

Figure 3.2.2 Average nitrogen contents of wood blocks during the soil burial study.

Figure 3.2.3 Dehydrogenase activity in the outer wood surface of untreated softwood blocks during the soil burial study.

Figure 3.2.4 Dehydrogenase activity in the outer wood surface of lime blocks during the soil burial study.

Figure 3.2.5 Dehydrogenase activity in the inner wood of untreated softwood blocks during the soil burial study.

Figure 3.2.6 Dehydrogenase activity in the inner wood of lime blocks during the soil burial study.

Figure 3.2.7 Dehydrogenase activity in soil adjacent to pine blocks during the soil burial study.

Figure 3.2.8 Dehydrogenase activity in soil adjacent to spruce blocks during the soil burial study.

Figure 3.2.9 Dehydrogenase activity in soil adjacent to lime blocks during the soil burial study.

Figure 3.2.10 Average dehydrogenase activity in soil adjacent to the wood blocks during the soil burial study.

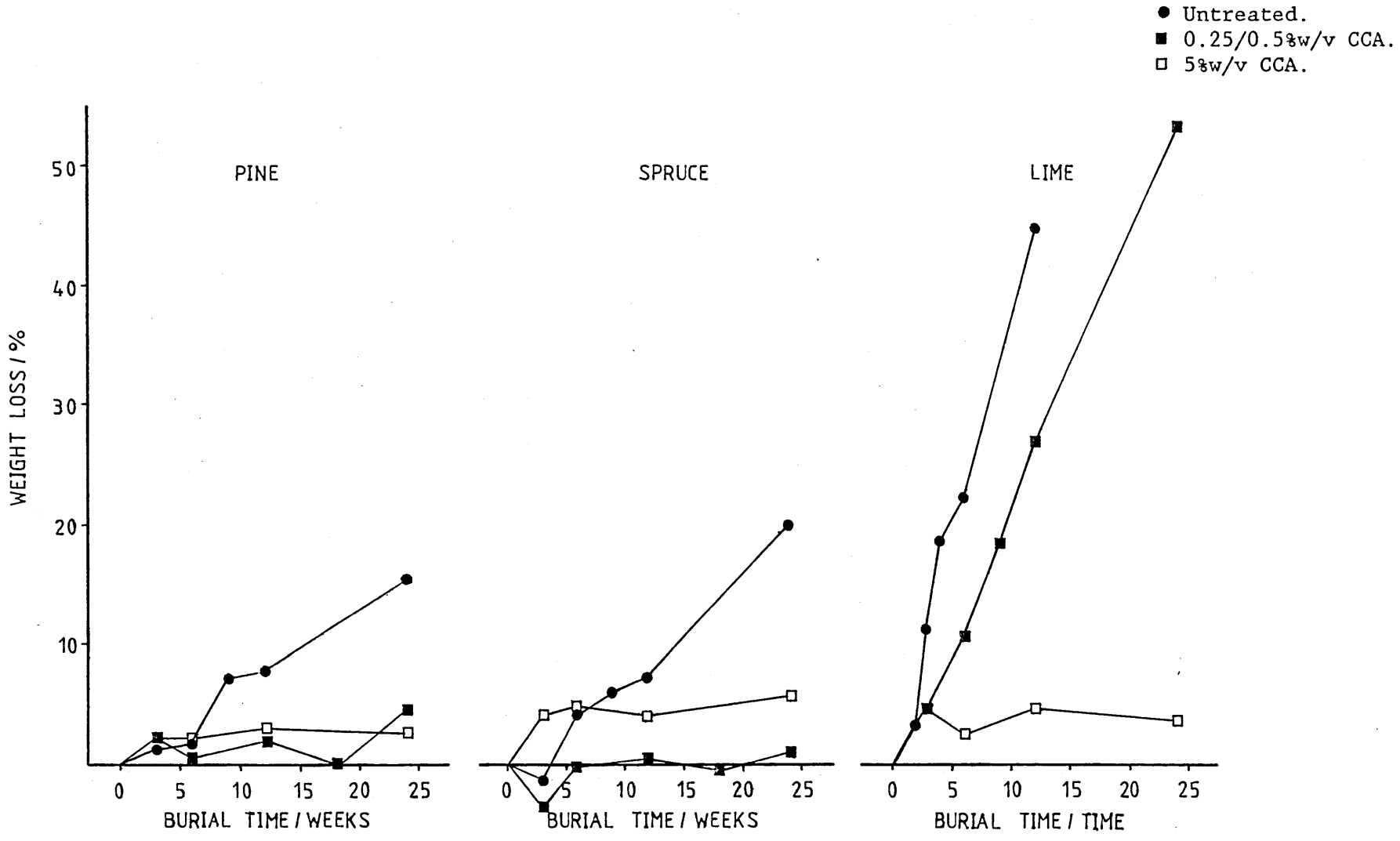


Figure 3.2.1 Average weight losses of wood blocks during the soil burial study. Average is based on 4 replicates.

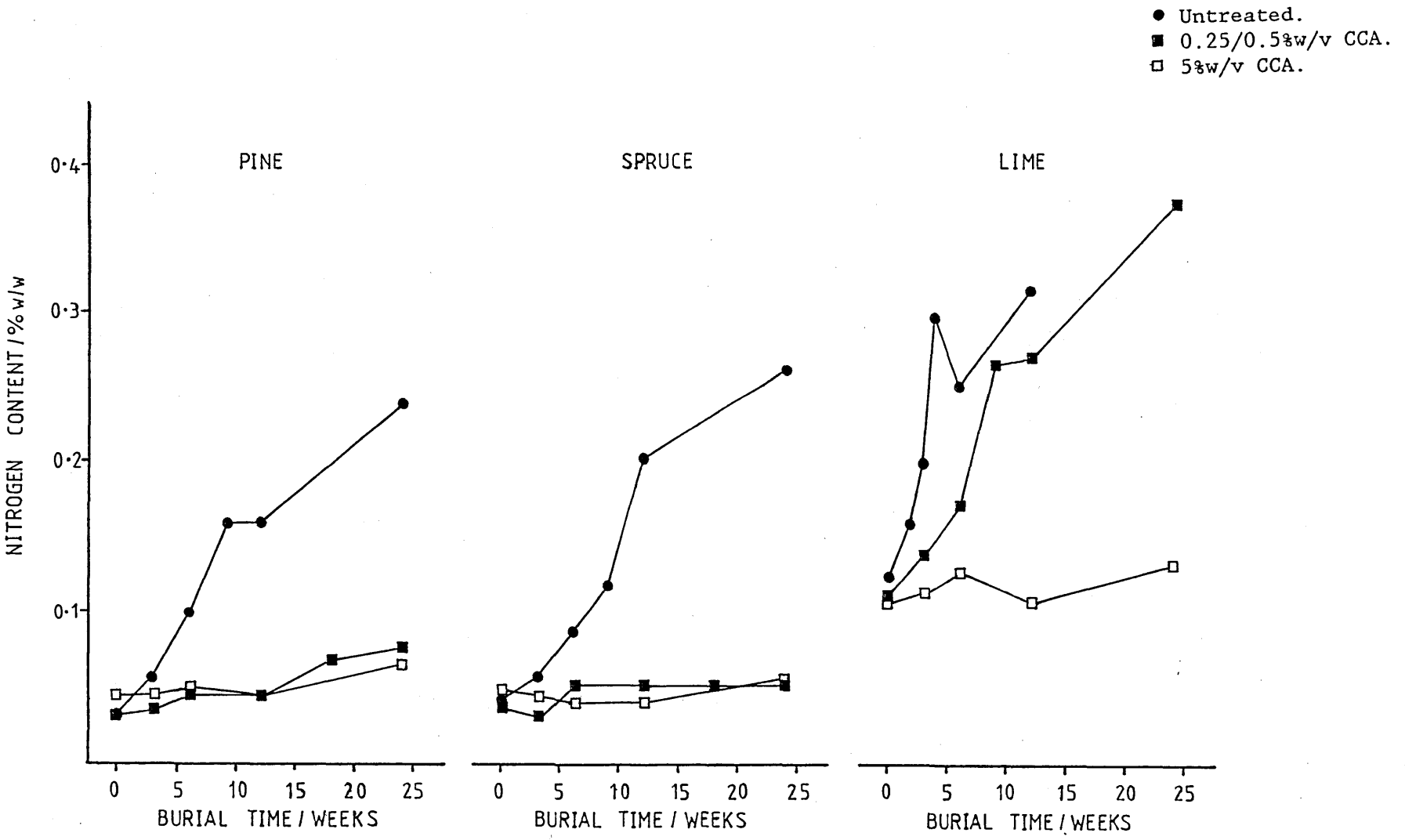


Figure 3.2.2 Average nitrogen contents of wood blocks during the soil burial study. Average is based on 4 replicates.

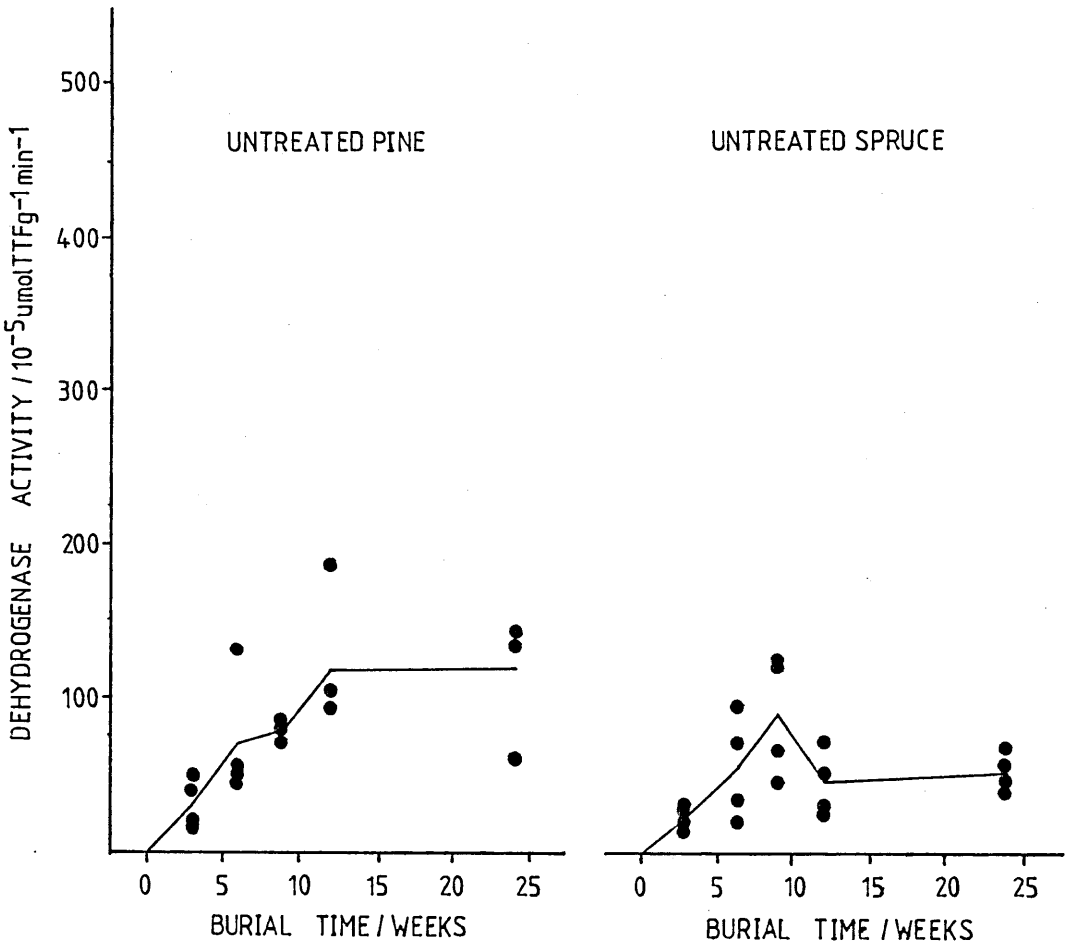


Figure 3.2.3 Dehydrogenase activity in the outer wood surface of untreated softwood blocks during the soil burial study. Individual results are shown and a curve is drawn through the average levels. Average is base on 4 replicates.

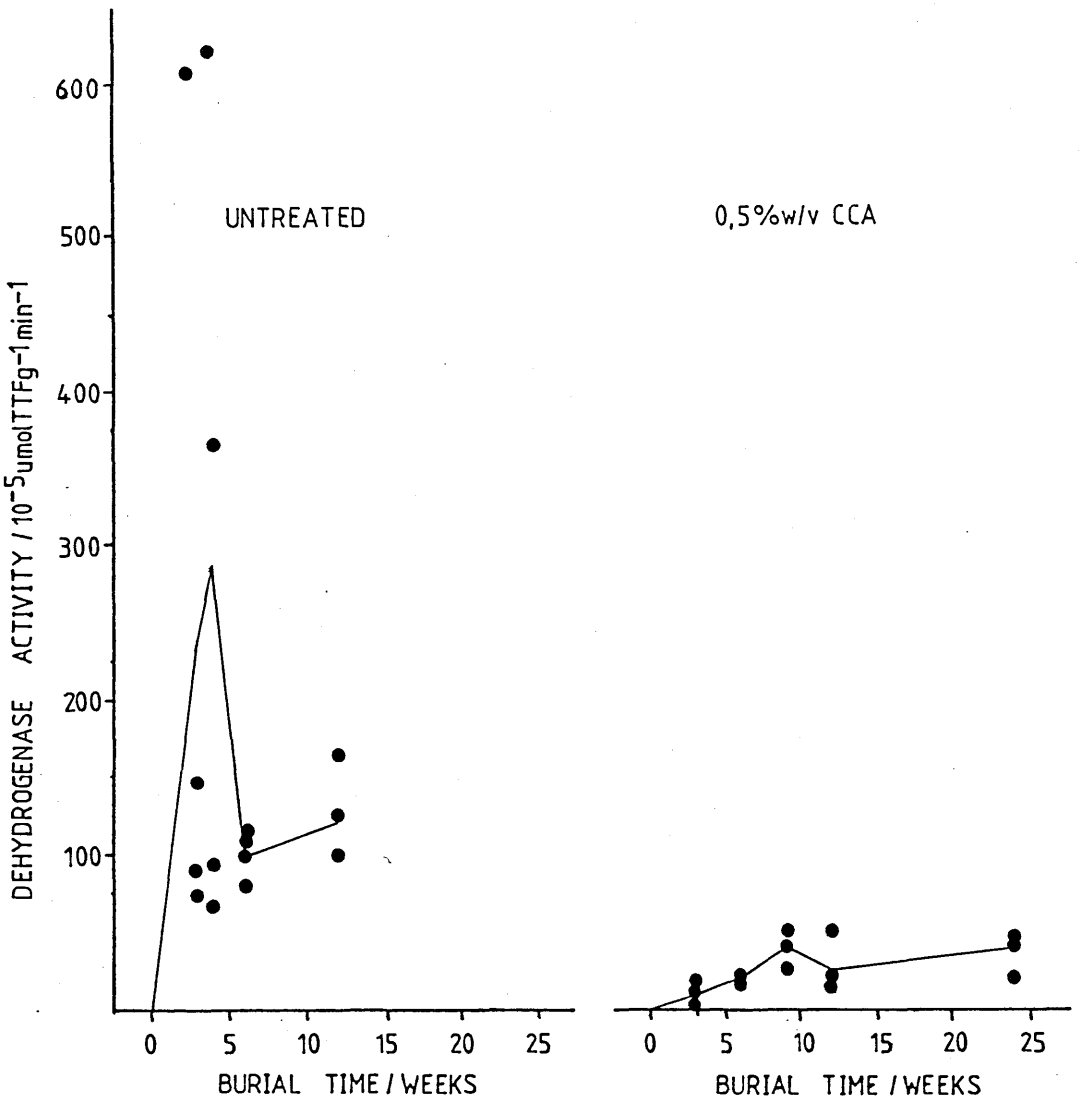


Figure 3.2.4 Dehydrogenase activity in the outer wood surface of lime blocks during the soil burial study. Individual results are shown and a curve is drawn through the average levels. Average is base on 4 replicates.

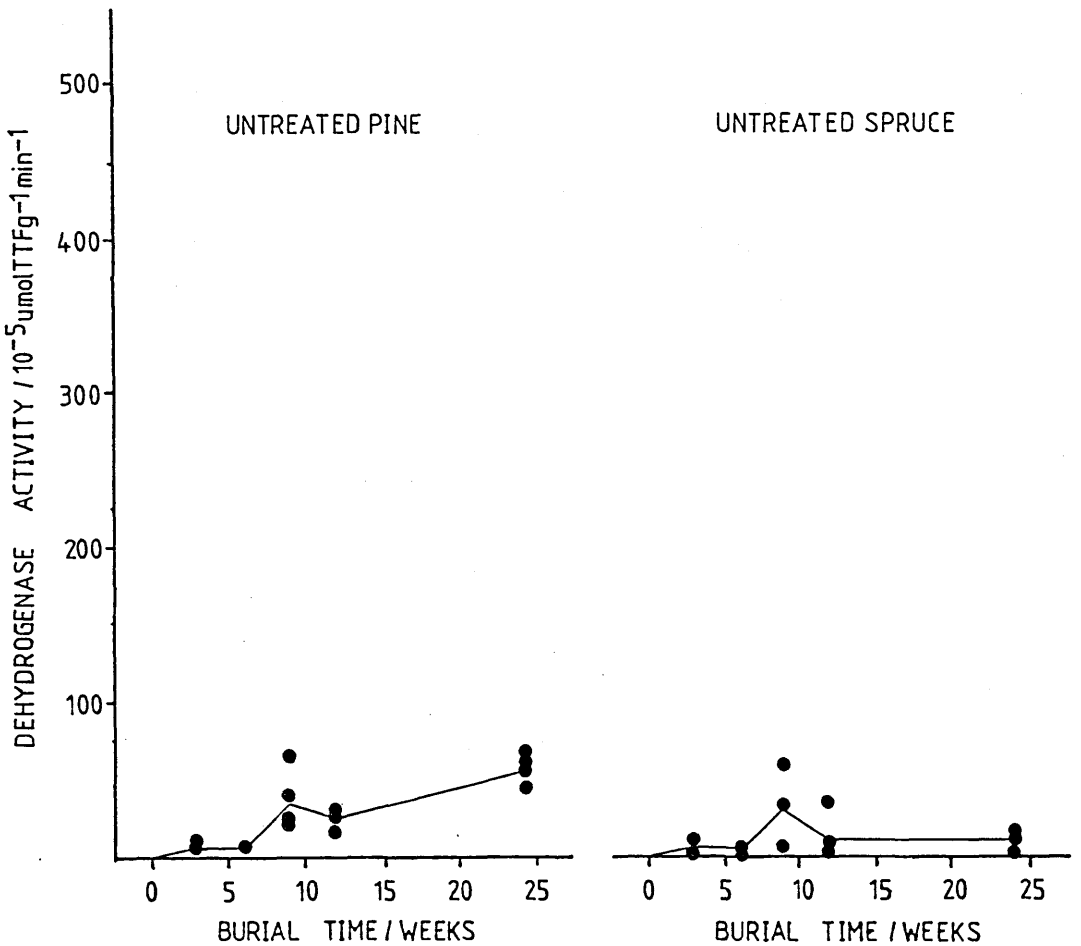


Figure 3.2.5 Dehydrogenase activity in the inner wood of untreated softwood blocks during the soil burial study. Individual results are shown and a curve is drawn through the average levels. Average is base on 4 replicates.

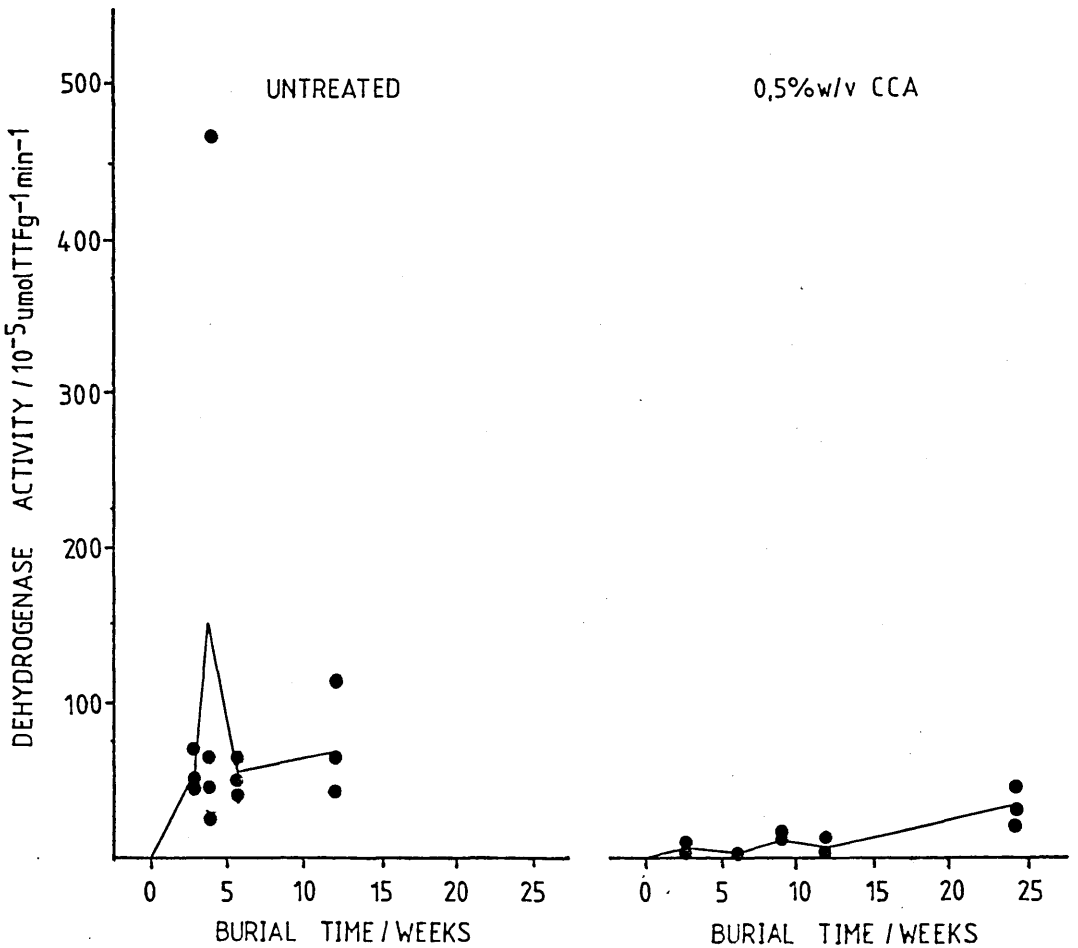


Figure 3.2.6 Dehydrogenase activity in the inner wood of lime blocks during the soil burial study. Individual results are shown and a curve is drawn through the average levels. Average is base on 4 replicates.

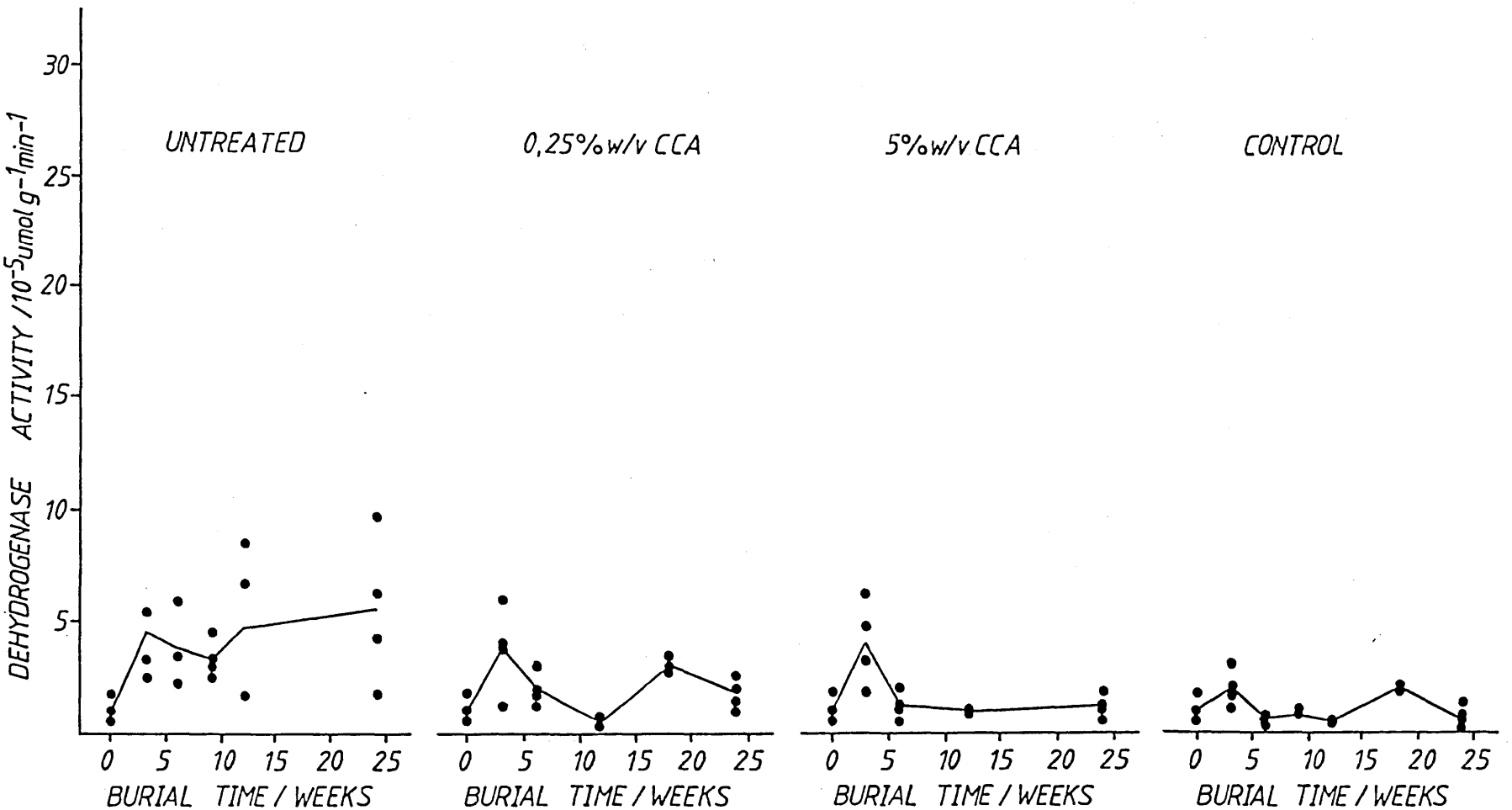


Figure 3.2.7 Dehydrogenase activity in soil adjacent to pine blocks during the soil burial study. Individual results are shown and a curve is drawn through the average levels. Average is base on 4 replicates.

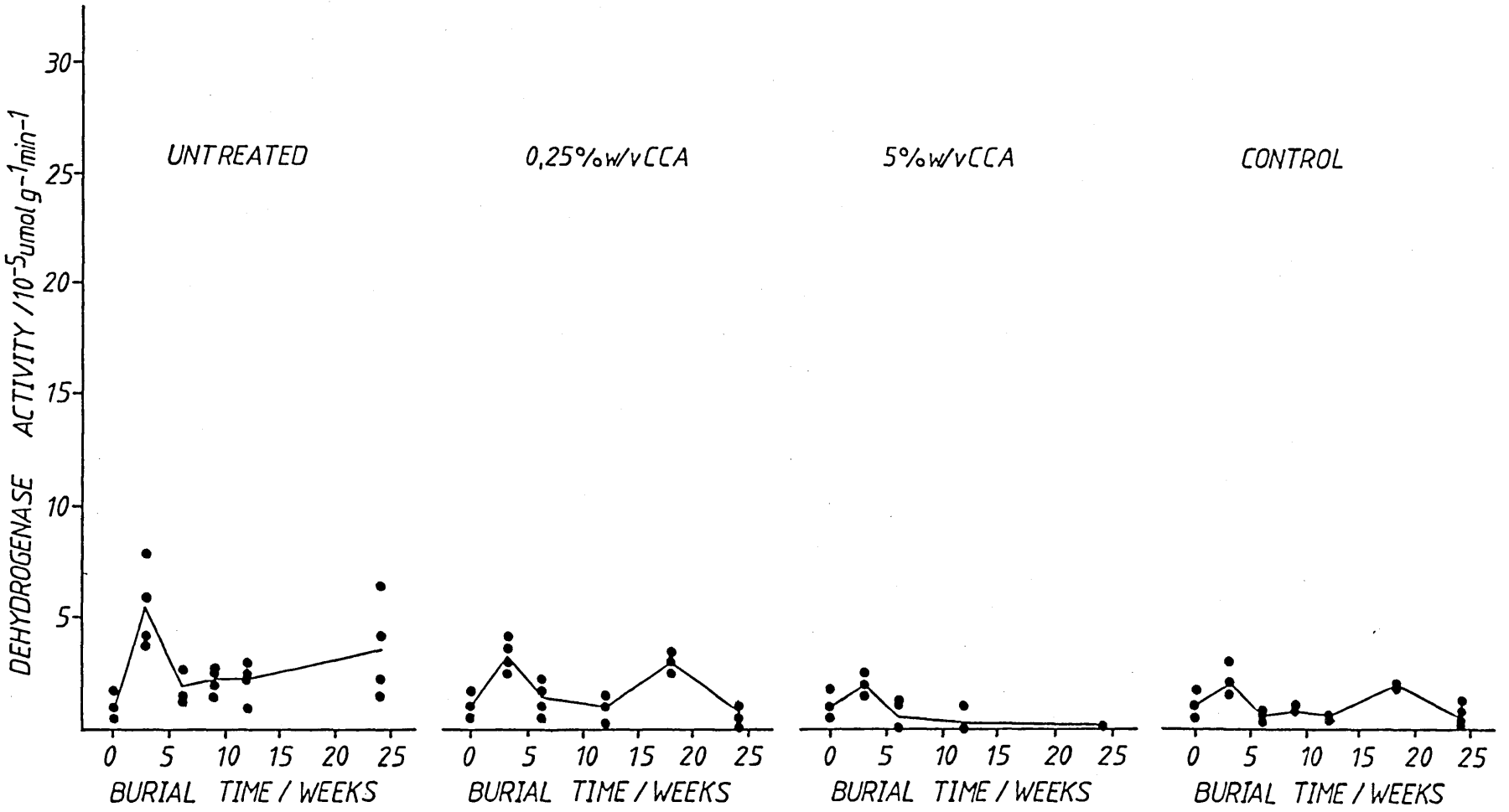


Figure 3.2.8 Dehydrogenase activity in soil adjacent to spruce blocks during the soil burial study. Individual results are shown and a curve is drawn through the average levels. Average is base on 4 replicates.

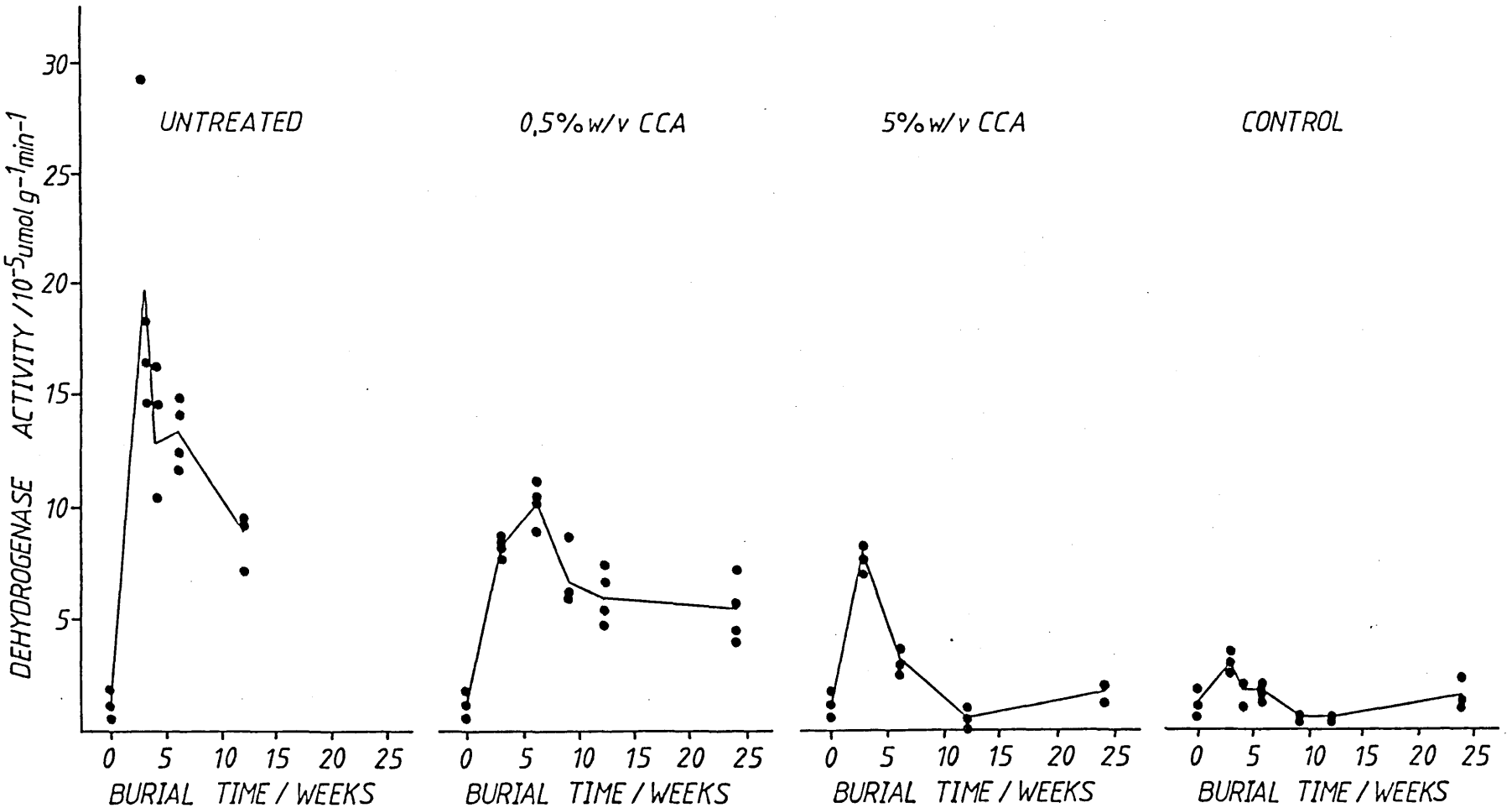


Figure 3.2.9 Dehydrogenase activity in soil adjacent to lime blocks during the soil burial study. Individual results are shown and a curve is drawn through the average levels. Average is base on 4 replicates.

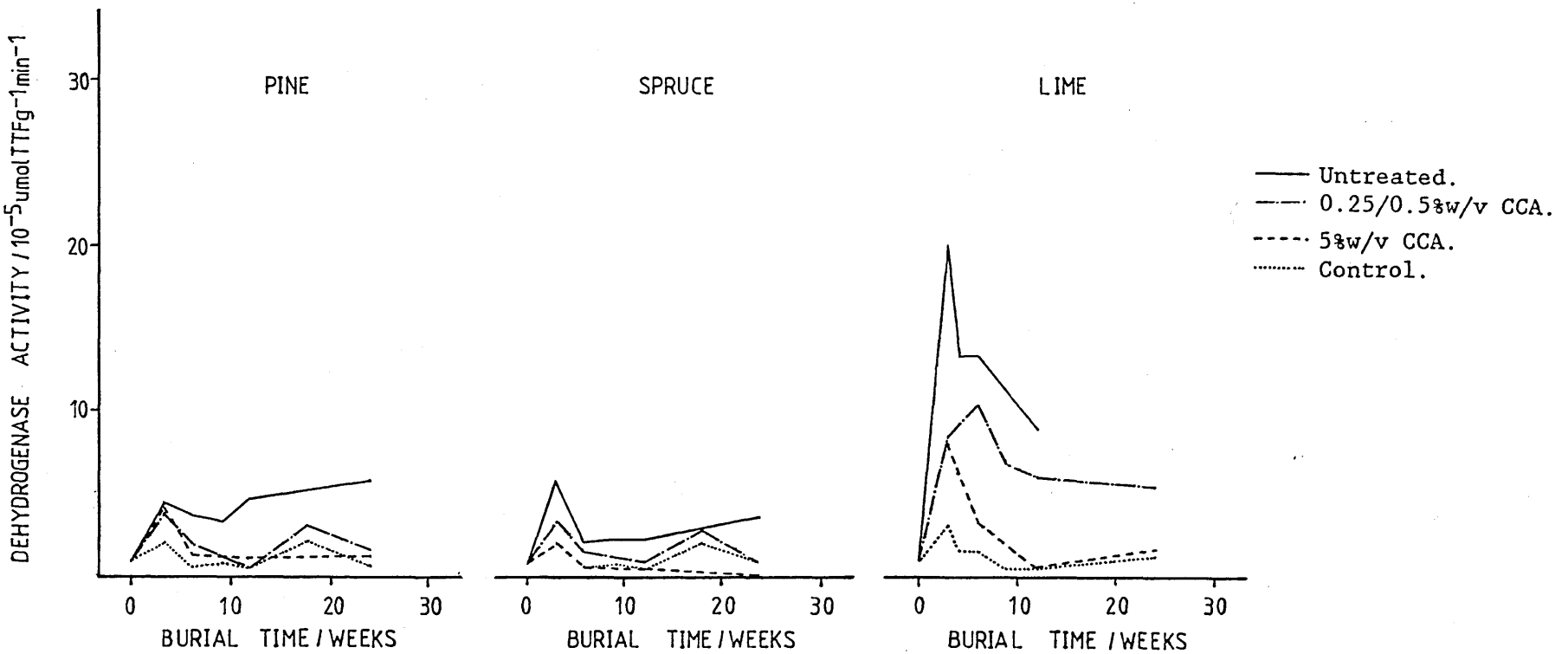


Figure 3.2.10 Average dehydrogenase activity in soil adjacent to the wood blocks during the soil burial study. Average is base on 4 replicates.

3.2.9 Tables 3.2.1-3.2.18.

Table 3.2.1 Moisture contents of softwood blocks.

Table 3.2.2 Moisture contents of lime blocks.

Table 3.2.3 Weight losses of softwood blocks.

Table 3.2.4 Weight losses of lime blocks.

Table 3.2.5 Estimated time of initiation of microbial decay.

Table 3.2.6 Estimated rate of decay.

Table 3.2.7 Softwood nitrogen contents during the soil burial study.

Table 3.2.8 Statistical comparison of softwood nitrogen contents during the soil burial study.

Table 3.2.9 Nitrogen contents of lime blocks during the study.

Table 3.2.10 Statistical comparison of nitrogen contents for lime blocks during the soil burial study.

Table 3.2.11 Estimated nitrogen content at time of initiation of microbial decay.

Table 3.2.12 Dehydrogenase activity in the outer wood surface of softwood blocks during the soil burial study.

Table 3.2.13 Dehydrogenase activity in the outer wood surface of lime blocks during the soil burial study.

Table 3.2.14 Dehydrogenase activity in the inner wood of softwood blocks during the soil burial study.

Table 3.2.15 Dehydrogenase activity in the inner wood of lime blocks during the soil burial study.

Table 3.2.16 Dehydrogenase activity in soil adjacent to softwood blocks and in soil at a distance from the wood blocks.

Table 3.2.17 Dehydrogenase activity in soil adjacent to lime blocks and in soil at a distance from the wood blocks.

Table 3.2.18 Results of the experimental check on the dehydrogenase method.

Table 3.2.1 Moisture contents of softwood blocks during the soil burial study. Mean results ± standard deviations are presented (mean is based on 4 replicates).

Wood species	Burial time (weeks)	Moisture content (%w/w)		
		Untreated	0.25%w/v CCA	5%w/v CCA
Pine	3	138.6± 8.8	54.5±16.1	56.4± 5.2
	6	117.5±11.9	52.3± 5.5	49.9± 4.9
	9	137.8±12.4	M/N	M/N
	12	178.3± 9.6	60.8±10.0	53.4± 3.5
	18	M/N	123.5±14.1	M/N
	24	206.8±25.0	74.9± 3.9	49.9± 5.5
Spruce	3	133.4±37.9	49.3± 3.5	92.1±12.2
	6	164.4±63.0	51.7± 9.0	99.4±23.4
	9	128.8±28.8	M/N	M/N
	12	264.8±26.9	53.2± 3.8	91.2±16.6
	18	M/N	65.5± 4.1	M/N
	24	296.7±34.0	54.9± 7.8	91.9± 9.0

Table 3.2.2 Moisture contents of lime blocks during the soil burial study. Mean results ± standard deviations are presented (mean is based on 4 replicates).

Wood species	Burial time (weeks)	Moisture content (%w/w)		
		Untreated	0.50%w/v CCA	5%w/v CCA
Lime	2	65.9± 4.7	M/N	M/N
	3	94.6±15.1	71.0± 3.7	80.8± 1.8
	4	113.1±30.1	M/N	M/N
	6	108.3±25.7	75.0± 6.6	68.4± 2.6
	9	M/N	138.6±12.5	M/N
	12	174.4±50.5	128.2±27.7	72.7± 4.8
	24	M/N	234.5±43.8	69.4± 2.1

Key. M/N measurement not carried out.

Table 3.2.3 Weight losses of softwood blocks during the soil burial study. Mean results ± standard deviations are presented (mean is based on 4 replicates).

Wood species	Burial time (weeks)	Weight loss (%)		
		Untreated	0.25%w/v CCA	5%w/v CCA
Pine	3	0.84±3.25	1.63±2.77	1.48±0.67
	6	1.42±1.22	0.21±2.83	2.26±0.67
	9	7.07±2.29	M/N	M/N
	12	7.57±3.47	1.63±1.48	3.01±1.45
	18	M/N	0.07±2.77	M/N
	24	15.60±4.61	4.53±2.08	2.49±0.93
Spruce	3	+1.52±3.84	+3.55±1.00	3.90±3.01
	6	3.96±4.48	+0.15±2.10	4.42±1.99
	9	5.74±2.34	M/N	M/N
	12	7.08±3.43	0.21±0.95	3.73±3.63
	18	M/N	+0.62±1.42	M/N
	24	20.00±9.11	0.78±3.23	5.45±1.69

Table 3.2.4 Weight losses of lime blocks during the soil burial study. Mean results ± standard deviations are presented (mean is based on 4 replicates).

Wood species	Burial time (weeks)	Weight loss (%)		
		Untreated	0.50%w/v CCA	5%w/v CCA
Lime	2	3.13±0.42	M/N	M/N
	3	11.21±2.05	4.44±1.75	4.62±1.24
	4	18.51±7.55	M/N	M/N
	6	22.13±6.47	10.62±3.18	2.29±1.80
	9	M/N	18.65±2.34	M/N
	12	44.35±9.29	27.09±6.87	4.30±3.07
	24	M/N	53.18±7.12	3.67±0.68

Key. M/N measurement not carried out.

Table 3.2.5 Estimated time of initiation of microbial decay (weeks).

Wood species	Treatment		
	Untreated	0.25/0.5%	5%w/v CCA
Pine	7.25	22.0	<3%
Spruce	5.50	<3%	2.00 (*)
Lime	2.00	2.00	2.00 (*)

Key. <3% average weight loss did not exceed 3% throughout this soil burial study.
(*) weight loss exceeded 3%, but considered unlikely to be due to microbial decay.

Table 3.2.6 Estimated rate of decay (%/week).

Wood species	Treatment		
	Untreated	0.25/0.5%	5%w/v CCA
Pine	0.75	0.76	N/A
Spruce	1.08	N/A	N/A
Lime	4.43	2.28	N/A

Key. N/A calculation not applicable.

Table 3.2.7 Softwood nitrogen contents during the soil burial study. Mean results ± standard deviations are presented (mean is based on 4 replicates).

Wood species	Burial time (weeks)	Nitrogen content (%w/w)		
		Untreated	0.25%w/v CCA	5%w/v CCA
Pine	Unburied	0.032±0.003	0.033±0.012	0.046±0.012
	3	0.057±0.007	0.034±0.015	0.045±0.006
	6	0.099±0.020	0.046±0.008	0.049±0.009
	9	0.159±0.030	M/N	M/N
	12	0.160±0.049	0.045±0.005	0.046±0.013
	18	M/N	0.069±0.007	M/N
	24	0.240±0.055	0.076±0.020	0.064±0.001
Spruce	Unburied	0.042±0.003	0.037±0.005	0.049±0.011
	3	0.056±0.017	0.030±0.016	0.045±0.018
	6	0.088±0.016	0.053±0.008	0.039±0.003
	9	0.118±0.019	M/N	M/N
	12	0.204±0.019	0.051±0.014	0.040±0.010
	18	M/N	0.052±0.011	M/N
	24	0.262±0.035	0.052±0.004	0.057±0.009

Key. M/N measurement not carried out.

Table 3.2.8 Statistical comparison (one-way analysis of variance) of softwood nitrogen contents during the soil burial study.

Wood species	Untreated	0.25%w/v CCA	5%w/v CCA
Pine	***	***	*
Spruce	***	*	NS

Key. NS No significant difference.
* Significant difference: probability of difference arising by chance is < 5%.
*** Significant difference: probability of difference arising by chance is < 0.5%.

Table 3.2.9 Nitrogen contents of lime blocks during the soil burial study. Mean results ± standard deviations are presented (mean is based on 4 replicates).

Wood species	Burial time (weeks)	Nitrogen content (%w/w)		
		Untreated	0.50%w/v CCA	5%w/v CCA
Lime	Unburied	0.126±0.018	0.113±0.006	0.107±0.015
	2	0.160±0.014	M/N	M/N
	3	0.200±0.054	0.138±0.015	0.114±0.015
	4	0.298±0.035	M/N	M/N
	6	0.252±0.043	0.171±0.025	0.129±0.012
	9	M/N	0.266±0.020	M/N
	12	0.316±0.021	0.273±0.046	0.108±0.010
	24	M/N	0.377±0.064	0.132±0.006

Key. M/N measurement not carried out.

Table 3.2.10 Statistical comparison (one-way analysis of variance) of nitrogen contents for lime blocks during the soil burial study.

Untreated	0.5%w/v CCA	5%w/v CCA
***	***	*

Key. As table 3.2.8.

Table 3.2.11 Estimated nitrogen content at time of initiation of microbial decay (%w/w).

Wood species	Treatment		
	Untreated	0.25/0.5%	5%w/v CCA
Pine	0.123	0.072	N/A
Spruce	0.083	N/A	N/A
Lime	0.160	0.128	N/A

Key. N/A calculation not applicable.

Table 3.2.12 Dehydrogenase activity in the outer wood surface of softwood blocks during the soil burial study. Mean results ± standard deviations are presented (mean is based on 4 replicates).

Wood species	Burial time (weeks)	Dehydrogenase activity (*10^{-5} umol TTF g^{-1} min^{-1})		
		Untreated	0.25%w/v CCA	5%w/v CCA
Pine	3	31.9±16.1	1.1± 1.4	N/R
	6	70.4±42.6	N/R	N/R
	9	79.9± 5.6	M/N	M/N
	12	122.4±43.2	N/R	N/R
	18	M/N	0.5± 0.9	M/N
	24	120.9±39.3	0.4± 0.6	N/R
Spruce	3	22.2± 8.2	N/R	N/R
	6	54.9±33.9	N/R	N/R
	9	88.0±38.2	M/N	M/N
	12	45.1±20.5	N/R	N/R
	18	M/N	N/R	M/N
	24	50.9±12.3	N/R	N/R

Table 3.2.13 Dehydrogenase activity in the outer wood surface of lime blocks during the soil burial study. Mean results ± standard deviations are presented (mean is based on 4 replicates).

Wood species	Burial time (weeks)	Dehydrogenase activity (*10^{-5} umol TTF g^{-1} min^{-1})		
		Untreated	0.50%w/v CCA	5%w/v CCA
Lime	3	233.0±256.0	8.1± 6.9	N/R
	4	289.1±265.0	M/N	M/N
	6	102.0± 15.9	18.3± 3.2	N/R
	9	M/N	41.0±12.0	M/N
	12	122.0± 31.3	26.5±16.2	N/R
	24	M/N	38.8±11.9	N/R

Key. M/N measurement not carried out.
N/R no reading obtained.

Table 3.2.14 Dehydrogenase activity in the inner wood of softwood blocks during the soil burial study. Mean results ± standard deviations are presented (mean is based on 4 replicates).

Wood species	Burial time (weeks)	Dehydrogenase activity (*10^{-5} umol TTF g^{-1} min^{-1})		
		Untreated	0.25%w/v CCA	5%w/v CCA
Pine	3	7.6± 3.2	N/R	N/R
	6	5.5± 1.0	N/R	N/R
	9	36.7±19.3	M/N	M/N
	12	23.3± 6.9	N/R	N/R
	18	M/N	N/R	M/N
	24	56.6± 8.9	N/R	N/R
Spruce	3	3.9± 4.6	N/R	N/R
	6	3.3± 2.5	N/R	N/R
	9	32.4±22.2	M/N	M/N
	12	12.1±15.6	N/R	N/R
	18	M/N	N/R	M/N
	24	8.4± 4.4	N/R	N/R

Table 3.2.15 Dehydrogenase activity in the inner wood of lime blocks during the soil burial study. Mean results ± standard deviations are presented (Mean is based on 4 replicates).

Wood species	Burial time (weeks)	Dehydrogenase activity (*10^{-5} umol TTF g^{-1} min^{-1})		
		Untreated	0.50%w/v CCA	5%w/v CCA
Lime	3	53.0±11.9	3.3±3.0	N/R
	4	152.0±215.0	M/N	M/N
	6	55.3±11.3	1.3±0.9	N/R
	9	M/N	11.1±1.2	M/N
	12	65.8±32.9	6.4±3.2	N/R
	24	M/N	34.0±8.2	N/R

Key. M/N measurement not carried out.
N/R no reading obtained.

Table 3.2.16 Dehydrogenase activity in soil adjacent to softwood blocks during the soil burial study, and in soil at a distance from the wood blocks. Mean results ± standard deviations are presented (mean is based on 4 replicates).

Wood species	Burial time (weeks)	Dehydrogenase activity ($*10^{-5}$ umol TTF g^{-1} min^{-1})			
		Untreated	0.25%w/v CCA	5%w/v CCA	Soil at a distance
Pine	3	4.4±1.5	3.7±1.9	4.0±2.0	1.9±0.8
	6	3.8±1.6	2.0±0.7	1.2±0.7	0.6±0.3
	9	3.3±0.8	M/N	M/N	0.8±0.2
	12	4.6±3.4	0.4±0.2	1.0±0.2	0.4±0.1
	18	M/N	3.0±0.2	M/N	2.0±0.2
	24	5.5±3.4	1.7±0.6	1.2±0.6	0.6±0.5
Spruce	3	5.5±2.0	3.3±0.7	2.1±0.5	1.9±0.8
	6	2.0±0.7	1.4±0.8	0.6±0.7	0.6±0.3
	9	2.2±0.6	M/N	M/N	0.8±0.2
	12	2.2±0.8	0.9±0.5	0.3±0.6	0.4±0.1
	18	M/N	3.0±0.4	M/N	2.0±0.2
	24	3.6±2.2	0.7±0.4	0.1±0.1	0.6±0.5

Table 3.2.17 Dehydrogenase activity in soil adjacent to lime blocks during the soil burial study, and in soil at a distance from the wood blocks. Mean results ± standard deviations are presented (Mean is based on 4 replicates).

Wood species	Burial time (weeks)	Dehydrogenase activity ($*10^{-5}$ umol TTF g^{-1} min^{-1})			
		Untreated	0.50%w/v CCA	5%w/v CCA	Soil at a distance
Lime	3	19.9±6.7	8.3±0.5	7.9±0.6	3.0±0.3
	4	13.1±3.1	M/N	M/N	1.7±0.4
	6	13.4±1.5	10.3±0.8	3.2±0.5	1.7±0.3
	9	M/N	6.8±1.3	M/N	0.4±0.2
	12	8.9±1.0	6.1±1.2	0.5±0.5	0.3±0.1
	24	M/N	5.4±1.3	1.7±0.3	1.4±0.7

Key. M/N measurement not carried out.
N/R no reading obtained.

Table 3.2.18 Results of the experimental check on the dehydrogenase method. Mean absorbances ± standard deviations (mean is based on a minimum of 5 replicates), along with percentage recoveries, are presented.

Sample	Rinsing technique	Control absorbance	Test absorbance	Recovery (%)
Soil	I	1.019±0.019	0.906 0.030	88.9
Untreated pine	II	0.099 0.002	0.086 0.006	86.8
Untreated spruce	I	0.088 0.077	0.082 0.009	92.9
Untreated lime	I	1.019 0.019	0.944 0.047	92.6
0.25%w/v CCA-treated pine	I	0.099 0.002	0.053 0.008	53.3
0.25%w/v CCA-treated spruce	I	0.088 0.077	0.050 0.008	57.4
3%w/v CCA-treated lime	I	1.019 0.019	0.632 0.153	62.0
5%w/v CCA-treated spruce	I	0.088 0.077	0.023 0.004	26.7
0.07%w/v ACA-treated spruce	II	0.101 0.014	0.039 0.003	38.6
0.14%w/v ACA-treated lime	II	0.099 0.002	0.056 0.007	56.6
1.41%w/v ACA-treated spruce	II	0.101 0.014	0.022 0.009	22.0

3.3 Experimental programme 3.

Chemical and microbiological studies on the effect of ammonia on the wood-soil system.

3.3.1. Cold water leach study.

3.3.1.1 Introduction.

The extent of copper, arsenic and nitrogen losses from 1.41%w/v ACA-treated blocks during leaching were assessed as described in section 3.1.1.1.

3.3.1.2 Copper, arsenic and nitrogen contents of daily leach liquors.

Distilled water containing $6.85*10^{-3} \pm 1.65*10^{-3}$ ug cm^{-3} of copper, $1.18*10^{-3} \pm 2.03*10^{-3}$ ug cm^{-3} of arsenic and no nitrogen was used to leach the ACA-treated wood blocks.

The copper, arsenic and nitrogen contents of the individual, duplicate daily leach liquors employed in leaching the ACA-treated blocks are shown in figures 3.3.1.1-3.3.1.3.

For each wood species the copper contents of the duplicate leach liquors were very similar (figure 3.3.1.1). The highest and lowest amounts of copper were detected in leach liquors from lime and spruce blocks respectively (figure 3.3.1.1). During the 5 days of leaching the greatest amounts of copper were measured after the first day (figure 3.3.1.1); between 60 and 85% of the total copper measured in the liquors during the 5 days of leaching was measured on the first day.

The arsenic contents of the individual daily leach liquors are shown in figure 3.3.1.2. Note that the scale of the y-axis used is a

tenth of that in figure 3.3.1.1. For each wood species, the levels of arsenic in the individual leach liquors were generally similar, with the exception of the first day for spruce (figure 3.3.1.2). The greatest amount of arsenic measured on any day was on the first day for all wood species (figure 3.3.1.2); 71-76% of the total arsenic detected during 5 days of leaching was measured on the first day. The amounts of arsenic measured in the daily leach liquors were substantially greater than corresponding levels of copper (figures 3.3.1.2 and 3.3.1.1).

The nitrogen contents of the individual daily leach liquors are shown in figure 3.3.1.3. For each wood species, levels of nitrogen in the individual leach liquors were comparable during this study (figure 3.3.1.3). The largest amounts of nitrogen were measured in leach liquors from the ACA-treated lime blocks (figure 3.3.1.3), though levels in the pine leach liquors were only slightly lower, while levels in spruce leach liquors were considerably lower.

As with copper and arsenic (figures 3.3.1.1 and 3.3.1.2), the highest levels of nitrogen were measured after the first day of leaching for all three wood species (figure 3.3.1.3). The amounts of nitrogen measured after the first day accounted for between 75 and 85% of the total amounts of nitrogen measured during the experiment. After the first day, only minute quantities of nitrogen were measured in the liquors.

3.3.1.3 Copper, arsenic and nitrogen contents of ACA-treated wood blocks.

The copper, arsenic and nitrogen contents of the ACA-treated wood blocks are presented in table 3.3.1.1. The average contents of the unleached and leached blocks were compared to assess the extent of

their losses during leaching, as described in section 3.1.1.1; the results of these comparisons are presented in table 3.3.1.2. F and T-tests were carried out on these data (section 2.6); the results of these statistical analyses are also presented in table 3.3.1.2.

The average copper contents of unleached and leached, 1.41%w/v ACA-treated pine and lime blocks were very similar (table 3.3.1.1). The average arsenic content of the leached spruce blocks was 10% lower than the unleached blocks. However, for all three wood species statistical analyses indicated no significant reduction in the copper contents of the ACA-treated blocks had occurred (table 3.3.1.2).

For all three wood species, the average arsenic contents of the leached blocks were considerably lower than those of their unleached counterparts (table 3.3.1.1). The difference between the average arsenic concentrations of the unleached and leached blocks suggests that after 5 days of leaching over 50% of the arsenic in the softwood blocks was lost to the leaching water (table 3.3.1.2), while losses from the ACA-treated lime blocks were in excess of 65%. Differences were also seen between the average nitrogen contents of the unleached and leached ACA-treated wood blocks (table 3.3.1.1). These differences indicate between 58 and 68% of the nitrogen in the ACA-treated blocks prior to leaching was lost to the leaching water (table 3.3.1.2), with slightly greater losses occurring from the softwood blocks than from lime.

The differences between the average arsenic and nitrogen contents of the unleached and leached, 1.41%w/v ACA-treated blocks were so clear-cut that statistical analyses was not really required. However, F and T-tests were carried out. For spruce and lime T-tests were not justified for either the arsenic or the nitrogen data (table 3.3.1.2). T-tests were able to be carried out on the arsenic and

nitrogen data of the pine blocks; these indicated highly significant ($p < 0.5\%$) losses of these two elements had taken place (table 3.3.1.2).

3.3.1.4 Leach losses of copper, arsenic and nitrogen (%) from ACA-treated wood blocks as calculated by the addition method.

Percentage losses of copper, arsenic and nitrogen were calculated using the addition method, as previously described (section 3.1.1.1). The results of these calculations are presented in table 3.3.1.3.

Similar percentage losses of copper occurred from the ACA-treated softwood blocks during this experiment, though larger losses of copper were measured for the lime blocks (table 3.3.1.3). However, all copper losses were relatively small when compared with losses of arsenic and nitrogen from these blocks (table 3.3.1.3).

The greatest percentage losses of any of the three elements were of arsenic from the ACA-treated pine and lime blocks (table 3.3.1.4). Percentage losses of arsenic from ACA-treated spruce blocks were lower than those for the other two wood species when losses were determined by this method. For all three wood species percentage losses of arsenic determined by the addition method were greater than those determined by the comparison method (tables 3.3.1.2 and 3.3.1.3). In contrast, percentage losses of nitrogen determined by both methods were very similar for all wood species (tables 3.3.1.2 and 3.3.1.3). Greater percentage leach losses of nitrogen occurred from the softwoods than from the lime blocks (table 3.3.1.3).

3.3.1.5 Figures 3.3.1.1-3.3.1.3.

Figure 3.3.1.1 Copper contents of daily leach liquors from 1.41%w/v ACA-treated wood blocks during the leaching experiment.

Figure 3.3.1.2 Arsenic contents of daily leach liquors from 1.41%w/v ACA-treated wood blocks during the leaching experiment.

Figure 3.3.1.3 Nitrogen contents of daily leach liquors from 1.41%w/v ACA-treated wood blocks during the leaching experiment.

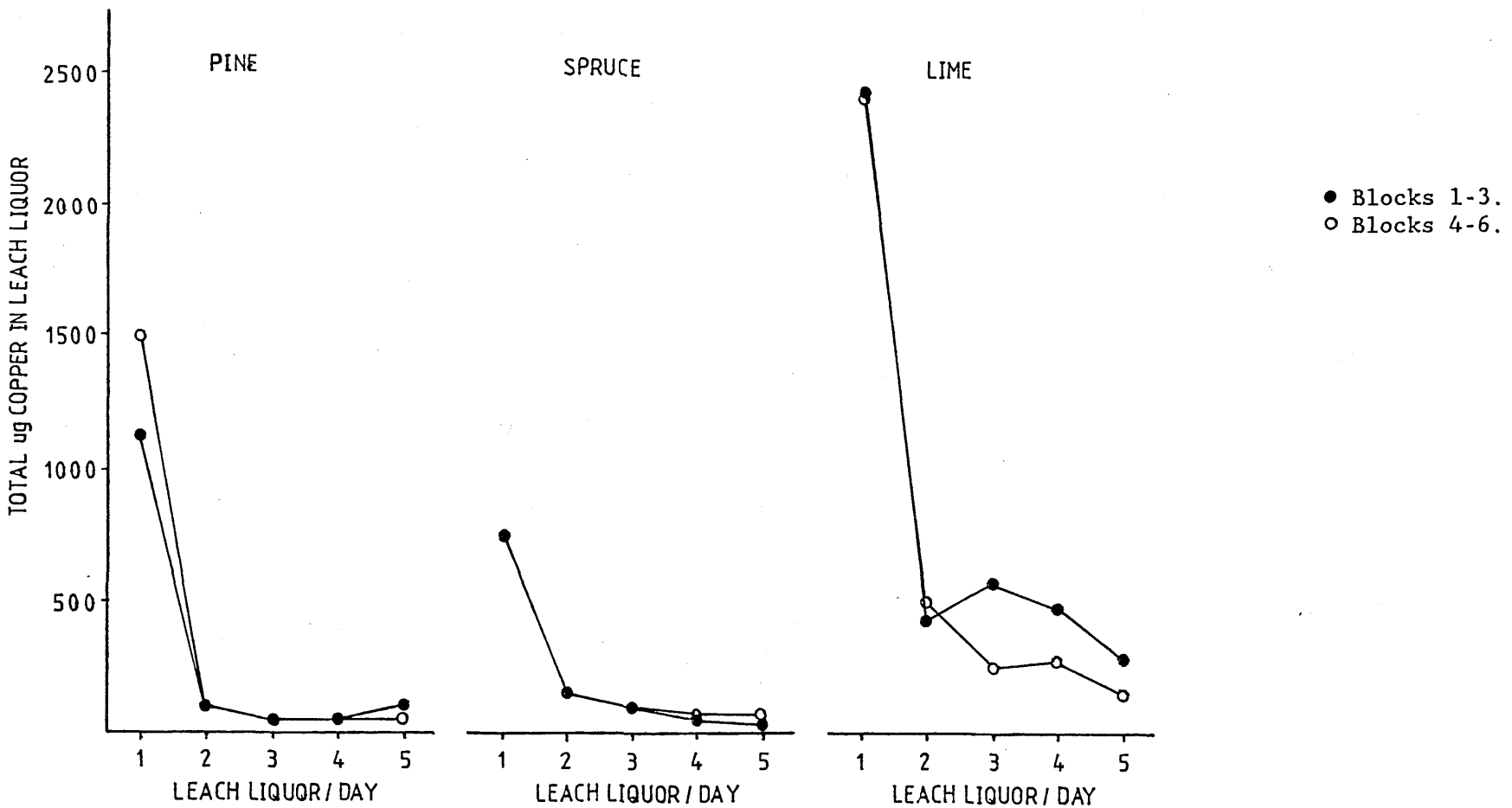


Figure 3.3.1.1 Copper contents of daily leach liquors from 1.41%w/v ACA-treated wood blocks during the leaching experiment.

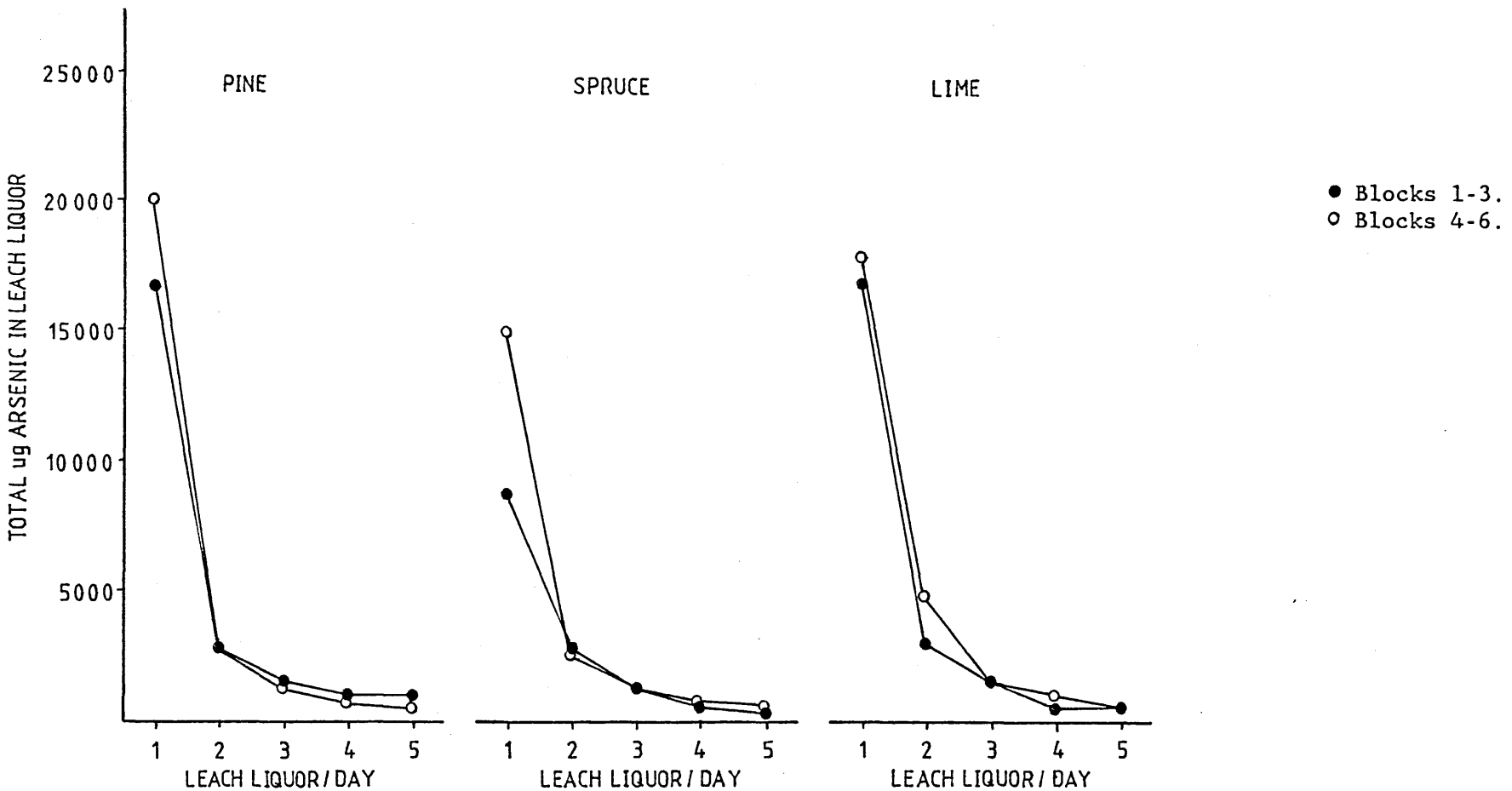


Figure 3.3.1.2 Arsenic contents of daily leach liquors from 1.41%w/v ACA-treated wood blocks during the leaching experiment.

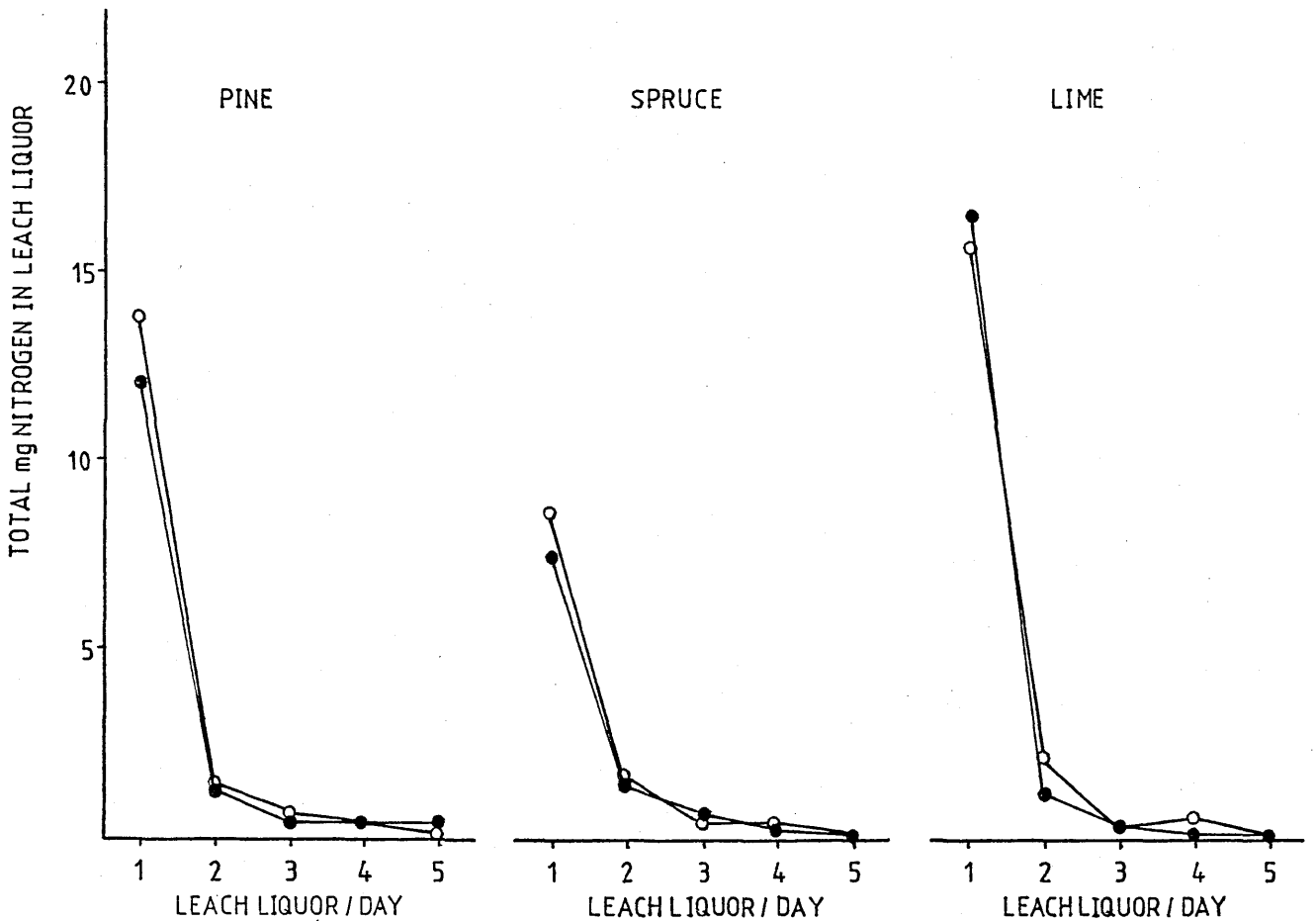


Figure 3.3.1.3 Nitrogen contents of daily leach liquors from 1.41%w/v ACA-treated wood blocks during the leaching experiment.

Blocks 1-3. ●
Blocks 4-6. ○

3.3.1.6 Tables 3.3.1.1-3.3.1.3.

Table 3.3.1.1 Copper, arsenic and nitrogen contents of ACA-treated wood blocks.

Table 3.3.1.2 Leach losses (%) of copper, arsenic and nitrogen from ACA-treated wood blocks, as calculated by the comparison method. T-test results are also presented.

Table 3.3.1.3 Leach losses (%) of copper, arsenic and nitrogen from ACA-treated wood blocks, as calculated by the addition method.

Table 3.3.1.1 Copper, arsenic and nitrogen contents of ACA-treated wood blocks. Mean results ± standard deviations are presented (for unleached blocks mean is based on 4 replicates, for leached blocks mean is based on a minimum of 5 replicates).

Wood species	Leaching	Element (%w/w)		
		Copper	Arsenic	Nitrogen
Pine	Not leached	0.667±0.042	0.413±0.022	0.537±0.010
	Leached	0.679±0.056	0.195±0.043	0.188±0.005
Spruce	Not leached	1.207±0.134	1.011±0.240	0.595±0.056
	Leached	1.070±0.172	0.459±0.063	0.190±0.019
Lime	Not leached	0.577±0.109	0.352±0.152	0.836±0.113
	Leached	0.579±0.056	0.120±0.024	0.348±0.040

Table 3.3.1.2 Leach losses (%) of copper, arsenic and nitrogen from 6 ACA-treated wood blocks during a 5 day cold water leach experiment, as calculated by the comparison method. T-test results are also presented.

Wood species	Copper		Arsenic		Nitrogen	
	Loss	T-test	Loss	T-test	Loss	T-test
Pine	Inc	NS	52.8	***	65.0	***
Spruce	11.3	NS	54.6	⁋	68.1	⁋
Lime	Inc	NS	65.9	⁋	58.4	⁋

Key Inc Mean metal content of leached blocks is greater than that of unleached blocks.
⁋ F-test indicates that a T-test cannot be carried out.
NS No significant difference.
*** Significant difference: probability of the difference arising by chance is < 0.5%.

Table 3.3.1.3 Leach losses (%) of copper, arsenic and nitrogen from 6 ACA-treated wood blocks during a 5 day cold water leach experiment, as calculated by the addition method.

Wood species	Total losses of element (%)		
	Copper	Arsenic	Nitrogen
Pine	5.3	74.8	66.5
Spruce	3.8	58.0	67.7
Lime	13.2	81.9	55.0

3.3.2.1 Soil burial study.

Two-way analysis of variance (see section 2.6) was used to assess the effect of ammonia on wood moisture content and weight loss. The effect of ammonia on dehydrogenase activity in wood and adjacent soil was also investigated by this method. One and two-way analyses of variance were used to assess changes in nitrogen levels of the buried wood blocks and also preservative metal levels in soil adjacent to the buried blocks. Losses of preservative metals from the blocks were estimated by the comparison method (section 3.1.1.1), using preservative metals data for unburied blocks and for blocks buried for 18 weeks. Results of these calculations and statistical analyses are presented in the appropriate sections.

3.3.2.2 Moisture contents of buried wood blocks.

Average wood moisture contents and standard deviations are presented in tables 3.3.2.1 (softwoods) and 3.3.2.2 (lime). Data for untreated and ammonia-treated blocks of each wood species were compared using the two-way analysis of variance; the results are given in table 3.3.2.3.

All wood moisture contents recorded in this study were in excess of the fibre saturation point (tables 3.3.2.1 and 3.3.2.2). However, the moisture contents of the untreated blocks were lower in this study than previously measured (tables 3.1.2.1, 3.1.3.1 and 3.2.1). It should be noted that this was the only soil burial study where the soil used was sieved by a mechanical siever. Furthermore, the moisture content of the soil used here was 21%w/w at 100% of its water holding capacity, which was lower than obtained in previous studies (approximately 23%w/w, Appendix 2).

The moisture contents of all untreated and ammonia-treated wood blocks increased during the study (tables 3.3.2.1 and 3.3.2.2). The moisture contents of ammonia-treated blocks were equal to or greater than those of their untreated counterparts. Treatment with ammonia did not significantly affect the uptake of moisture by softwood blocks (table 3.3.2.3). However, ammonia-treated lime blocks had significantly greater ($p < 5\%$) moisture contents than the untreated lime blocks (table 3.3.2.3).

The moisture contents of all ACA-treated softwood blocks remained approximately constant during the study (table 3.3.2.1). This was also the case for the 1.41%w/v ACA-treated lime blocks. However, the moisture contents of the 0.14%w/v ACA-treated lime blocks increased throughout the study (table 3.3.2.2). Before significant decay of the untreated and ammonia-treated wood blocks was measured (see section 3.3.2.3), the moisture contents of these blocks and the ACA-treated blocks were similar (tables 3.3.2.1 and 3.3.2.2).

3.3.2.3 Weight loss of wood blocks.

Average weight losses and standard deviations for each replicate group of wood blocks are presented in tables 3.3.2.4 (softwoods) and 3.3.2.5 (lime). The average weight losses are also shown in figure 3.3.2.1. The results of two-way analysis of variance to investigate the effect of ammonia-treatment on weight loss are presented in table 3.3.2.6. The time at which decay of the blocks was initiated and the subsequent rate of decay of these blocks were estimated as described in section 3.1.2.2; the results are given in tables 3.3.2.7 and 3.3.2.8 respectively.

The weight loss of all untreated wood blocks was in excess of 3% by the conclusion of this study (figure 3.3.2.1). While the weight

losses of untreated pine and spruce blocks were similar, resulting in similar decay rates (table 3.3.2.8), decay of the pine blocks began more rapidly (table 3.3.2.7). Of the untreated blocks decay began most rapidly in the lime blocks (table 3.3.2.7) as well as proceeding at the greatest rate (table 3.3.2.8).

Significant weight loss was measured in the ammonia-treated pine blocks earlier than untreated pine blocks (table 3.3.2.7). The ammonia-treated pine blocks also decayed more rapidly than untreated pine blocks (figure 3.3.2.1, table 3.3.2.8). Despite these differences there was no significant difference between the weight losses of the ammonia and untreated pine blocks (table 3.3.2.6).

During the first 12 weeks of this experiment there was little difference in the average weight losses of the ammonia and untreated spruce blocks. Consequently decay was estimated to have begun in both groups of wood blocks at the same time (table 3.3.2.7). However, at final burial time the average weight loss of the ammonia-treated spruce blocks was greater than that of the untreated blocks (figure 3.3.2.1). Thus, the decay rate of the ammonia-treated spruce blocks was also greater (table 3.3.2.8). Ammonia treatment was found to significantly ($p < 0.5\%$) increase the weight loss of the spruce blocks.

Decay began in the ammonia-treated lime blocks slightly more rapidly than in the untreated lime blocks (figure 3.3.2.1, table 3.3.2.7). The ammonia-treated lime blocks also decayed more rapidly than any group of wood blocks in this study (table 3.3.2.8). Ammonia treatment significantly increased the microbial decay of the lime blocks, since a highly significant interaction mean square ($p < 0.5\%$) was obtained (table 3.3.2.6).

Decay was estimated to have started slightly earlier in the 0.14%w/v ACA-treated lime blocks than in either the untreated or

ammonia-treated lime blocks (table 3.3.2.7). Up to the 6 week sampling time the average weight losses of the 0.14%w/v ACA-treated lime blocks were always greater than those for the untreated lime blocks (figure 3.3.2.1). However, they were less than the average weight loss of the ammonia-treated lime blocks. By the 12 week burial time there was a significant reduction in the rate of weight loss of the 0.14%w/v ACA-treated lime blocks (figure 3.3.2.1). Average weight losses of these blocks were less in the latter part of the study than those of the untreated lime blocks. Consequently the rate of weight loss of these preservative treated lime blocks over the entire burial period was slightly less than the untreated lime blocks (table 3.3.2.8).

Average weight loss results of the 0.07 and 1.41%w/v ACA-treated softwood blocks were always less than 3% (figure 3.3.2.1), indicating no decay of these blocks had occurred. 1.41%w/v ACA-treated lime blocks had an average weight loss greater than 3% at every time interval and weight loss increased throughout the experiment (figure 3.3.2.1). However, there was no surface discolouration of these blocks. Furthermore, there was no increase in their wood nitrogen contents (see section 3.3.2.4) indicating that microbial decay of the 1.41%w/v CCA-treated lime blocks was probably not occurring.

3.3.2.4 Wood block nitrogen contents.

The nitrogen contents of the wood blocks from this soil burial study are presented in tables 3.3.2.9 and 3.3.2.10 and are shown in figures 3.3.2.2-3.3.2.4. To determine whether there had been a significant increase in the nitrogen content of the buried blocks, a one-way analysis of variance was carried out. However, from figures 3.3.2.2-3.3.2.4 it was evident that a substantial decrease in the

nitrogen contents of the ammonia and ACA-treated blocks occurred soon after their emplacement in soil. Including this data in the statistical analysis would have resulted in highly statistically significant decreases being measured for the ammonia and ACA-treated blocks. Thus any subsequent increases in the nitrogen contents of these blocks in the remainder of the study would have been masked. Therefore, the nitrogen contents of all unburied wood blocks were omitted from the statistical analysis. The results of the statistical analysis are given in table 3.3.2.11. The nitrogen contents of the wood blocks at the time that soft rot decay was initiated were determined as described in section 3.1.2.3; the results are presented in table 3.3.2.12.

The average nitrogen contents of all untreated wood blocks exhibited general increases during this soil burial study (figures 3.3.2.2-3.3.2.4). All the increases observed in the untreated wood blocks were highly significant ($p < 0.5\%$).

The average nitrogen contents of all ammonia and ACA-treated wood blocks decreased rapidly on soil burial (figures 3.3.2.2-3.3.2.4). However, the average nitrogen contents of these wood blocks were still greater at the first sampling interval than those of the equivalent untreated wood blocks.

Following the initial decrease in their nitrogen contents, the levels of nitrogen in ammonia-treated wood blocks of all three wood species increased throughout the study. The increases in the nitrogen contents of the ammonia-treated spruce and lime blocks were highly significant ($p < 0.5\%$, table 3.3.2.11). The increase in the nitrogen contents of the ammonia-treated pine blocks were only moderately significant ($p < 1\%$, table 3.3.2.11). This may be due to the relatively large standard deviations obtained for these blocks at the last two sampling intervals (figure 3.3.2.2).

The 0.14%w/v ACA-treated lime blocks were the only group of preservative treated wood blocks for which a substantial increase in wood nitrogen contents was measured (figure 3.3.2.4). This increase was highly significant ($p < 0.5\%$, table 3.3.2.11)

There was a small increase in the nitrogen contents of the 1.41%w/v ACA-treated softwood blocks in the latter part of this study (figures 3.3.2.2 and 3.3.2.3), which was slightly significant ($p <$ 5%, table 3.3.2.11). No increases in the nitrogen contents of the 0.07%w/v ACA-treated softwood blocks and the 1.41%w/v ACA-treated lime blocks were measured.

Wood blocks decayed during the soil burial study all exhibited highly significant nitrogen content increases during the experiment. Small increases in the nitrogen contents of the untreated softwood blocks were observed before significant weight loss was measured (tables 3.3.2.9, 3.3.2.12). However, for all other decayed wood blocks no increase in wood nitrogen contents was evident when decay began (tables 3.3.2.9, 3.3.2.10 and 3.3.2.12).

For each wood species the nitrogen contents of the unburied blocks increased in the order,

untreated < ammonia < 0.07/0.14%w/v ACA < 1.41%w/v ACA,

The decreases in the wood nitrogen contents of the ammonia and ACA-treated wood blocks, which occurred by the first sampling interval did not affect this order. The nitrogen contents of the unburied, ammonia and ACA-treated lime blocks were substantially greater than those of the equivalent untreated lime blocks. This result was also obtained for these wood blocks at the first sampling interval.

3.3.2.5 Nitrogen as ammonia contents of the wood blocks.

Results are expressed in terms of concentrations of nitrogen, rather than ammonia, to allow a direct comparison with those in section 3.3.2.4.

Average nitrogen as ammonia contents and their standard deviations are given in tables 3.3.2.13 and 3.3.2.14, and average values are also shown in figure 3.3.2.5. The nitrogen as ammonia contents of ammonia and ACA-treated wood blocks sampled in the early stages of the soil burial experiment were relatively consistent (figure 3.3.2.5). Therefore the determination of nitrogen as ammonia contents of buried wood blocks was discontinued after the 3 and 6 week burial times for the lime and softwood blocks respectively.

To assess the significance of the nitrogen as ammonia levels, the extent of the rapid losses of both nitrogen and nitrogen as ammonia on burial were calculated. The loss of nitrogen as ammonia was then expressed as a percentage of the nitrogen loss. The results of these determinations are presented in table 3.3.2.15. The extent of the "additional" nitrogen observed in the buried ammonia and ACA-treated blocks was also calculated. These results were compared with the average nitrogen as ammonia contents of blocks sampled at the first burial time (table 3.3.1.16).

For each wood species the nitrogen as ammonia contents of the unburied block increased in the order,

untreated < ammonia < 0.07/0.14%w/v ACA < 1.41%w/v ACA

(tables 3.3.2.13 and 3.3.2.14). The nitrogen as ammonia contents of the unburied, 1.41%w/v ACA-treated pine and lime blocks were only slightly greater than those of the 0.07 and 0.14%w/v ACA-treated pine and lime blocks (tables 3.3.2.13 and 3.3.2.14). However, for ACA-treated spruce blocks the difference was relatively large. There

was always a substantial difference between the nitrogen as ammonia contents of the ammonia-treated blocks and the ACA-treated blocks (tables 3.3.2.13 and 3.3.2.14). However, nitrogen concentrations in the ammonia-treated blocks were still very much greater than those of the untreated blocks.

All ammonia and ACA-treated wood blocks lost between 80 and 90% of their nitrogen as ammonia content by the first sampling interval (figure 3.3.2.5), after which the nitrogen as ammonia contents remained approximately constant. There were greater levels of nitrogen as ammonia in the buried, ammonia and ACA-treated blocks than in the untreated blocks. The levels in the buried blocks increased with the treatment, as described for the unburied wood blocks, though differences were less obvious (tables 3.3.2.13 and 3.3.2.14). Buried, ammonia and ACA-treated pine blocks had similar nitrogen as ammonia contents to the comparably treated spruce blocks. However, levels in the lime blocks were substantially greater (figure 3.3.2.5).

The amounts of nitrogen lost from the ammonia and ACA-treated wood blocks soon after their emplacement in soil (see section 3.3.2.4) were compared with the decrease observed in the nitrogen as ammonia levels of the same blocks (table 3.3.2.15). The decrease in the nitrogen as ammonia contents of the blocks accounted for between 79 and 102% of the decrease in the block nitrogen contents.

Buried ammonia and ACA-treated blocks had greater nitrogen and nitrogen as ammonia contents than the untreated blocks (section 3.3.2.4, tables 3.3.2.13 and 3.3.2.14). However, the nitrogen as ammonia contents of the buried wood blocks accounted for less than 50% of the additional nitrogen present in these blocks (table 3.3.2.16).

3.3.2.6 Copper and arsenic contents of the wood blocks.

The preservative metal contents of all ACA-treated wood blocks were calculated from the liquid uptake data; the results are presented in tables 3.3.2.17 (copper) and 3.3.2.18 (arsenic). The copper and arsenic contents of unburied, untreated and ACA-treated wood blocks of all three wood species were determined by chemical analysis; the results are given in tables 3.3.2.19 (copper) and 3.3.2.21 (arsenic). Preservative metal levels in blocks sampled after 18 weeks are also presented in these tables. Losses of copper and arsenic from the ACA-treated wood blocks were calculated by the comparison method (see section 3.1.1.1). F and T-tests were also carried out. The calculated losses and the results of the statistical analyses are presented in tables 3.3.2.20 (copper) and 3.3.2.22 (arsenic).

The copper contents of all ACA-treated wood blocks were lower by the conclusion of the study than before soil burial (tables 3.3.2.19 and 3.3.2.20). However, the decreases in the copper contents of all 1.41%w/v ACA-treated wood blocks and of the 0.07%w/v ACA-treated spruce blocks were not significant. The copper contents of the 0.07%w/v ACA-treated pine blocks and the 0.14%w/v ACA-treated lime blocks were reduced by about 20% after 18 weeks of soil burial. These reductions were significant ($p < 1\%$ and $p < 5\%$ respectively, table 3.3.2.20).

By the comparison method the 0.07%w/v ACA-treated softwood blocks lost up to 65% of their original arsenic contents after 18 weeks of soil burial (table 3.3.2.22). However, these decreases were not significant. It should be noted that the variabilities within the replicate groups of arsenic determinations were frequently large (table 3.3.2.21), consequently affecting the results of the

statistical analysis.

Very little arsenic remained in the 0.14%w/v ACA-treated lime blocks by the conclusion of this study (table 3.3.2.21). Differences in the arsenic concentrations of the unburied and buried, 0.14%w/v ACA-treated lime blocks were so great a T-test could not be carried out (table 3.3.2.22).

Losses of arsenic of up to 60% were obtained for the 1.41%w/v ACA-treated lime and spruce blocks; these decreases were significant ($p < 5\%$, table 3.3.2.2). The average arsenic contents of the unburied and buried, 1.41%w/v ACA-treated pine were similar (table 3.3.2.21), indicating minimal arsenic losses. However, the variabilities within the two groups of data were very different; the standard deviation for the buried pine blocks being much greater than for the unburied wood blocks. Consequently a T-test was not justified to compare the average arsenic levels in the unburied and buried, 1.41%w/v ACA-treated pine blocks (table 3.3.2.22).

No significant changes in the copper and arsenic contents of the untreated wood blocks were measured (tables 3.3.2.20 and 3.3.2.22).

3.3.2.7 Levels of dehydrogenase activity in the outer wood surface of the wood blocks.

Average dehydrogenase activity levels in the outer wood surface of buried wood blocks are presented in tables 3.3.2.23 (softwoods) and 3.3.2.24 (lime); standard deviations are also given. Individual and average activities are shown in figures 3.3.2.6-3.3.2.8. Note that the scale of the y-axis in figure 3.3.2.8 is smaller than those used in figures 3.3.2.6 and 3.3.2.8.

Dehydrogenase levels in the outer surface of the untreated and ammonia-treated wood blocks were compared using a two-way analysis of

variance, although the variability of the data obtained (figures 3.3.2.8-3.3.2.10) casts doubt as to its validity. For all three wood species ammonia treatment did not affect the levels of activity. This was also the case for the inner wood and adjacent soil dehydrogenase activity levels.

Dehydrogenase activity was measured in all untreated and ammonia-treated pine and lime blocks (figures 3.3.2.6 and 3.3.2.8), with the exception of one ammonia-treated pine block sampled at the 3 week interval. Microbial activity was not measured in the untreated and ammonia-treated spruce blocks until after 3 weeks (figure 3.3.2.7). Activity levels in these spruce blocks were always lower than were measured in either of the other wood species.

Average dehydrogenase activity in the outer wood surface of the untreated pine blocks peaked after 12 weeks (figure 3.3.2.6). All dehydrogenase activities obtained for the untreated pine blocks at the 12 week sampling interval were well in excess of those measured 6 weeks earlier. However, activity levels in the ammonia-treated pine blocks were similar at the 6 and 12 week sampling times. Thus, after 6 weeks the activity levels in the ammonia-treated pine blocks were greater than those in the untreated blocks, though after 12 weeks they were similar. At the 18 week sampling time activity levels in both groups had fallen. The activities in the untreated and ammonia-treated pine blocks were similar by the final sampling time.

In untreated and ammonia-treated spruce blocks the greatest average levels of dehydrogenase activity and variability were measured after 12 weeks (figure 3.3.2.7). There were no obvious differences in the activity levels in the outer wood surfaces of the untreated and ammonia-treated spruce blocks.

After 1, 2 and 3 weeks dehydrogenase activity levels were greater in the outer wood surface of the ammonia-treated lime blocks than in

the untreated lime blocks (figure 3.3.2.8). Activity levels in both groups generally increased during the first 6 weeks, by which time the average level of activity was greater for the untreated lime blocks; this was also the case after 12 weeks. During the remainder of the experiment activity levels in both untreated and ammonia-treated lime blocks decreased significantly. Activity levels in these wood blocks were greater than those in any other group of blocks in this study.

Dehydrogenase activity was measured in all 0.14%w/v ACA-treated lime blocks (figure 3.3.2.8). Levels were relatively low during the first 3 weeks, however by the sixth week they had increased substantially, recording the maximum average level for these blocks. Activity fell during the remainder of the experiment, though levels were still greater than those measured during the first 3 weeks. Although activity levels measured in these wood blocks were low in comparison to other decaying lime blocks, they were greater than those in the untreated and ammonia-treated softwood blocks.

Occasional low levels of activity were measured in the 0.07%w/v ACA-treated pine blocks; the averages mean and standard deviations obtained are given in table 3.3.2.23. No dehydrogenase activity was measured in the outer wood surface of the 0.07%w/v ACA-treated spruce blocks or the 1.41%w/v ACA-treated blocks of all three wood species.

3.3.2.8 Levels of dehydrogenase activity in the inner wood of wood blocks.

Levels of dehydrogenase activity in the inner wood of blocks during this soil burial study are presented in tables 3.3.2.25 (softwoods) and 3.3.2.26 (lime). Individual and average levels measured in each block are shown in figures 3.3.2.9-3.3.2.11. Note

that the scale of the y-axis in figure 3.3.2.8 is smaller than those used in figures 3.3.2.6 and 3.3.2.8.

Activity was measured in the inner wood of all buried, untreated and ammonia-treated pine blocks (figure 3.3.2.9). Activity levels in these blocks increased during the first 12 weeks of soil burial. Levels measured at the 18 week burial time were similar to those measured 6 weeks earlier in both cases. At the 6 week burial time activity levels in the ammonia-treated blocks were greater than those in the untreated blocks sampled at the same time. There were no apparent differences in the activity levels in the inner wood of the two groups at any other burial time.

There was no measurable dehydrogenase activity in the inner wood of untreated and ammonia-treated spruce blocks until the twelfth week of the experiment (figure 3.3.2.10). Some dehydrogenase activity was measured in the untreated and ammonia-treated spruce blocks sampled at the 12 week burial time. However levels were low in comparison to those of the decaying pine blocks at this time (figure 3.3.2.9). After a further 6 weeks of burial there was little change in the dehydrogenase activity in the untreated spruce blocks, though levels in the ammonia-treated blocks had increased slightly.

Dehydrogenase activity was always measured in the inner wood of the untreated and ammonia-treated lime blocks (figure 3.3.2.11). Dehydrogenase activity levels in the ammonia-treated lime blocks were greater than in the untreated blocks at the 1, 2, 3 and 6 week burial times. The maximum average level of activity in the ammonia-treated wood blocks was measured at the 6 week burial time. Activity in these blocks fell slightly during the subsequent 6 weeks. Activity levels in the untreated lime blocks continued to increase up to the 12 week burial time, after which they fell slightly. Greater levels of dehydrogenase activity were measured in the inner wood of untreated

and ammonia-treated lime blocks than in any other decaying wood blocks in this study.

During the first 6 weeks of soil burial relatively low levels of dehydrogenase activity were measured in the inner wood of the 0.14%w/v ACA-treated lime blocks (figure 3.3.2.11). Activity had increased by the 12 week sampling time, when the greatest levels of dehydrogenase activity in these blocks were measured. During the final 6 weeks of the study there was some reduction in activity in these blocks. Activity levels measured in the inner wood of the 0.14%w/v ACA-treated lime blocks were similar to those measured for the inner wood of the untreated pine blocks (figure 3.3.2.9).

A low level of dehydrogenase activity was measured in one 0.07%w/v ACA-treated pine block. No dehydrogenase activity was measured in the inner wood of all other ACA-treated softwood blocks. Activity was also not detected in the 1.41%w/v ACA-treated lime blocks.

3.3.2.9 Levels of dehydrogenase activity in soil adjacent to buried wood blocks and in soil at a distance from these blocks.

Dehydrogenase activity in soil adjacent to the wood blocks is presented in tables 3.3.2.27 (softwoods) and 3.3.2.28 (lime). Levels of activity in soil at a distance from any buried wood blocks (controls) are also given in these tables. Individual and average levels are shown in figures 3.3.2.12-3.3.2.15.

Four replicate soil samples were assayed for dehydrogenase activity immediately before this experiment was set up; the level of activity was found to be $4.3 \pm 0.8 * 10^{-5}$ umol TTF g^{-1} min^{-1} . This level of activity represents the zero time level in all figures presented in this section.

Dehydrogenase activity in the hardwood control samples decreased during the first week (figure 3.3.2.14). However, after 2 and 3 weeks activity was greater than the zero time level. The activity subsequently returned to the pre-experiment level before increasing again at the final sampling time. Activity in the softwood control samples increased during the first 3 weeks of the study (figures 3.3.2.12 and 3.3.2.14) and subsequently fell remaining near the zero time level for the remainder of the experiment.

After 3 weeks dehydrogenase activity increased in the soil adjacent to all buried pine blocks (figure 3.3.2.12), particularly the untreated pine blocks (figure 3.3.2.15). Levels of activity in soil around ammonia and 0.07%w/w ACA-treated pine blocks were similar at the 3 week burial time and significantly greater than the control level. Increased activity in soil around 1.41%w/v ACA-treated pine blocks was only slightly greater than the control level. After 6 weeks dehydrogenase activity in all pine soil samples had fallen. Activity in soil adjacent to the untreated and ammonia-treated pine

blocks increased during the remainder of the study. Levels of activity for these wood blocks were similar after 6, 12 and 18 weeks. In soil adjacent to the 1.41%w/v ACA-treated pine blocks activity decreased to control levels by the 12 week burial time and remained at this level for the rest of the study. Following the decrease in soil activity around the 0.07%w/v ACA-treated pine blocks after 6 weeks, activities did not change significantly. The activity in soil adjacent to these blocks was always greater than the control levels.

By the 3 week burial time activity had increased in soil adjacent to all spruce blocks (figure 3.3.2.13). However, for each wood treatment the levels of activity in soil adjacent to the spruce blocks were lower than measured in the soil around the pine blocks (figure 3.3.2.15). At this sampling time the greatest levels of activity were measured in soil adjacent to the 0.07%w/v ACA-treated spruce blocks (figure 3.3.2.15), though levels for the untreated and ammonia-treated blocks were only slightly lower. Levels of activity in soil adjacent to 1.41%w/v ACA-treated spruce blocks were much lower than those around the other spruce blocks and were similar to the control levels.

After 6 weeks average levels of activity in the collected spruce soil samples were all reduced (figure 3.3.2.13). At this time activity in these soil samples increased in the order,

control/1.41%w/v ACA < 0.07%w/v < ammonia < untreated,

This order was maintained for the remainder of the experiment.

Activity levels in soil around the untreated spruce blocks increased greatly during the final 12 weeks of the study (figure 3.3.2.13). Increasing activity levels were also observed around the ammonia-treated spruce blocks. Levels of activity in soil adjacent to the 0.07%w/v ACA-treated spruce blocks remained at about their 6 week level for the rest of the study. Levels in soil around these blocks

were always significantly different from the control levels.

Dehydrogenase activities in soil adjacent to the 1.41%w/v ACA-treated spruce blocks were similar to the control levels for the majority of the study (figure 3.3.2.15). However, after 18 weeks activity around these blocks was slightly less than the control level.

Activity in soil adjacent to the lime blocks increased throughout the first three weeks (figure 3.3.2.14). For all treated lime blocks the greatest average soil activity level was measured at the 3 week burial time. However, the activity around untreated lime blocks continued to increase for a further 3 weeks. The maximum average activities in soil adjacent to all lime blocks were greater than levels in any softwood samples (figure 3.3.2.15). These maxima increased with the wood treatment in the order,

control < 1.41%w/v ACA < 0.14%w/v ACA < ammonia < untreated.

Activity levels around the ammonia-treated lime blocks were greater than those of the untreated blocks after 2 and 3 weeks. However, at the later sampling times greater activity levels were measured around the untreated lime blocks. A substantial decrease in activity around the untreated and ammonia-treated lime blocks followed the maximum activity. However, activities around these blocks were still greater than around the softwood blocks.

The maximum level of activity around the 0.14%w/v ACA-treated lime blocks was far lower than those of the untreated and ammonia-treated lime blocks (figure 3.3.2.14). However, the subsequent reduction in activity was also lower in soil adjacent to the 0.14%w/v ACA-treated lime blocks. In the latter part of the study levels around these blocks were similar to those of the untreated and ammonia-treated lime blocks (figure 3.3.2.15). Dehydrogenase activity in soil adjacent to the 1.41%w/v ACA-treated lime blocks decreased during the 3-18 week period. However, activity around these blocks

only approached the control level at the final sampling time.

3.3.2.10 Preservative metal levels in soil adjacent to buried wood blocks during the soil burial study.

Copper and arsenic concentrations (ug g^{-1} soil [dry weight]) of the soil used in this study were 42.18±0.98 and 18.15±19.69 respectively immediately before this experiment was set up. These metal levels were used in the statistical analyses in this section. The average levels are also shown on the appropriate graphs as the zero time data. Preservative metal concentrations of soil adjacent to untreated and ACA-treated blocks are given in tables 3.3.2.29-3.3.2.32. The average levels are also shown in figures 3.3.2.16 and 3.3.2.17. It should be noted that the scale on figure 3.3.2.17 is less than on figure 3.3.2.16.

Changes in the concentrations of copper and arsenic in soil adjacent to the buried wood blocks were investigated using the one-way analysis of variance (see section 3.2.6). Results of these statistical analyses are presented in table 3.3.2.33.

Soil preservative metal levels around the 1.41%w/v ACA-treated blocks were greater than concentrations in soil adjacent to the untreated wood blocks (figures 3.3.2.16 and 3.3.2.17). However, the differences between the soil metal concentrations for the less heavily treated blocks and the untreated blocks were less obvious. Therefore, two-way analysis of variance comparing the soil metal contents of the untreated and 0.07/0.14%w/v ACA-treated wood blocks was carried out. The results of these statistical analyses are presented in table 3.3.2.33.

The copper contents of soil adjacent to all ACA-treated wood blocks were greater than those around the untreated blocks (figure 3.3.2.16). The increases in copper levels observed were all highly significant ($p < 0.5\%$, table 3.3.2.33) and concentrations in soil adjacent to the 0.07/0.14%w/v ACA-treated wood blocks were significantly different from levels around the untreated blocks (table 3.3.2.34). Copper levels around the 1.41%w/v ACA-treated wood blocks were much greater than the concentrations adjacent to the less heavily treated blocks (figure 3.3.2.16). The copper concentrations of soil adjacent to the ACA-treated wood blocks generally continued to increase during the study.

The average copper levels of soil adjacent to the 1.41%w/v ACA-treated wood blocks of all three wood species were generally similar (figure 3.3.2.16). The average copper concentrations of soil collected from around the 0.07%w/v ACA-treated pine and spruce blocks were also similar throughout this study. However, copper levels in soil adjacent to the 0.14%w/v ACA-treated lime blocks were consistently greater than those of the 0.07%w/v ACA-treated softwood samples.

The average arsenic concentrations of soil adjacent to all 1.41%w/v ACA-treated blocks increased significantly ($p < 0.5\%$) during the study (figure 3.3.2.17, table 3.3.2.33). The greatest average arsenic concentrations were measured in soil adjacent to the 1.41%w/v ACA-treated lime blocks (figure 3.3.2.17). Of the soil in contact with the 1.41%w/v ACA-treated wood blocks, levels of arsenic were lowest around the spruce blocks.

The average arsenic levels were always greater in soil adjacent to 0.07%w/v ACA-treated softwood blocks than in soil around the untreated blocks (figure 3.3.2.17). One-way analysis of variance of the soil arsenic concentrations indicated no significant increase in

arsenic levels had occurred around these treated blocks (table 3.3.2.33). However, arsenic concentrations around the 0.07%w/v ACA-treated softwood blocks and the untreated blocks were significantly different ($p < 0.5\%$, table 3.3.2.34). This indicates an increase in arsenic levels in soil adjacent to the 0.07%w/v ACA-treated softwood blocks.

Average arsenic contents were greater around the 0.14%w/v ACA-treated lime blocks than in soil adjacent to the 0.07%w/v ACA-treated lime blocks (figure 3.3.2.17). The increase in arsenic concentration around these lime blocks was highly significant ($p < 0.5\%$, table 3.3.2.33). Arsenic contents of soil adjacent to the 0.14%w/v ACA-treated lime blocks were significantly different from levels in the soil around the untreated lime blocks (table 3.3.2.34).

There was no change in the arsenic concentrations of soil around the untreated wood blocks during this experiment (table 3.3.2.33) One-way analysis of variance on the soil copper levels of the untreated pine blocks indicated a moderately significant difference ($p < 1\%$, table 3.3.2.33). This difference is probably due to a low average copper concentration obtained for these blocks after 3 weeks of the study (figure 3.3.2.16). The copper contents of soil adjacent to the untreated spruce and lime blocks did not change significantly during this study (table 3.3.2.34).

3.3.2.11 Figures 3.3.2.1-3.3.2.17.

Figure 3.3.2.1 Average weight losses of wood blocks during the soil burial study

Figure 3.3.2.2 Mean nitrogen contents ± standard deviations of pine blocks during the soil burial study.

Figure 3.3.2.3 Mean nitrogen contents ± standard deviations of spruce blocks during the soil burial study.

Figure 3.3.2.4 Mean nitrogen contents ± standard deviations of lime blocks during the soil burial study.

Figure 3.3.2.5 Average nitrogen as ammonia contents of wood blocks during the soil burial study.

Figure 3.3.2.6 Dehydrogenase activity in the outer wood surface of pine blocks during the soil burial study.

Figure 3.3.2.7 Dehydrogenase activity in the outer wood surface of spruce blocks during the soil burial study.

Figure 3.3.2.8 Dehydrogenase activity in the outer wood surface of lime blocks during the soil burial study.

Figure 3.3.2.9 Dehydrogenase activity in the inner wood of pine blocks during the soil burial study.

Figure 3.3.2.10 Dehydrogenase activity in the inner wood of spruce blocks during the soil burial study.

Figure 3.3.2.11 Dehydrogenase activity in the inner wood of lime blocks during the soil burial study.

Figure 3.3.2.12 Dehydrogenase activity in soil adjacent to the pine blocks during the soil burial study.

Figure 3.3.2.13 Dehydrogenase activity in soil adjacent to the spruce blocks during the soil burial study.

Figure 3.3.2.14 Dehydrogenase activity in soil adjacent to the lime blocks during the soil burial study.

Figure 3.3.2.15 Average dehydrogenase activity in soil adjacent to the wood blocks during the soil burial study.

Figure 3.3.2.16 Average copper contents of soil adjacent to the wood blocks.

Figure 3.3.2.17 Average arsenic contents of soil adjacent to the wood blocks.

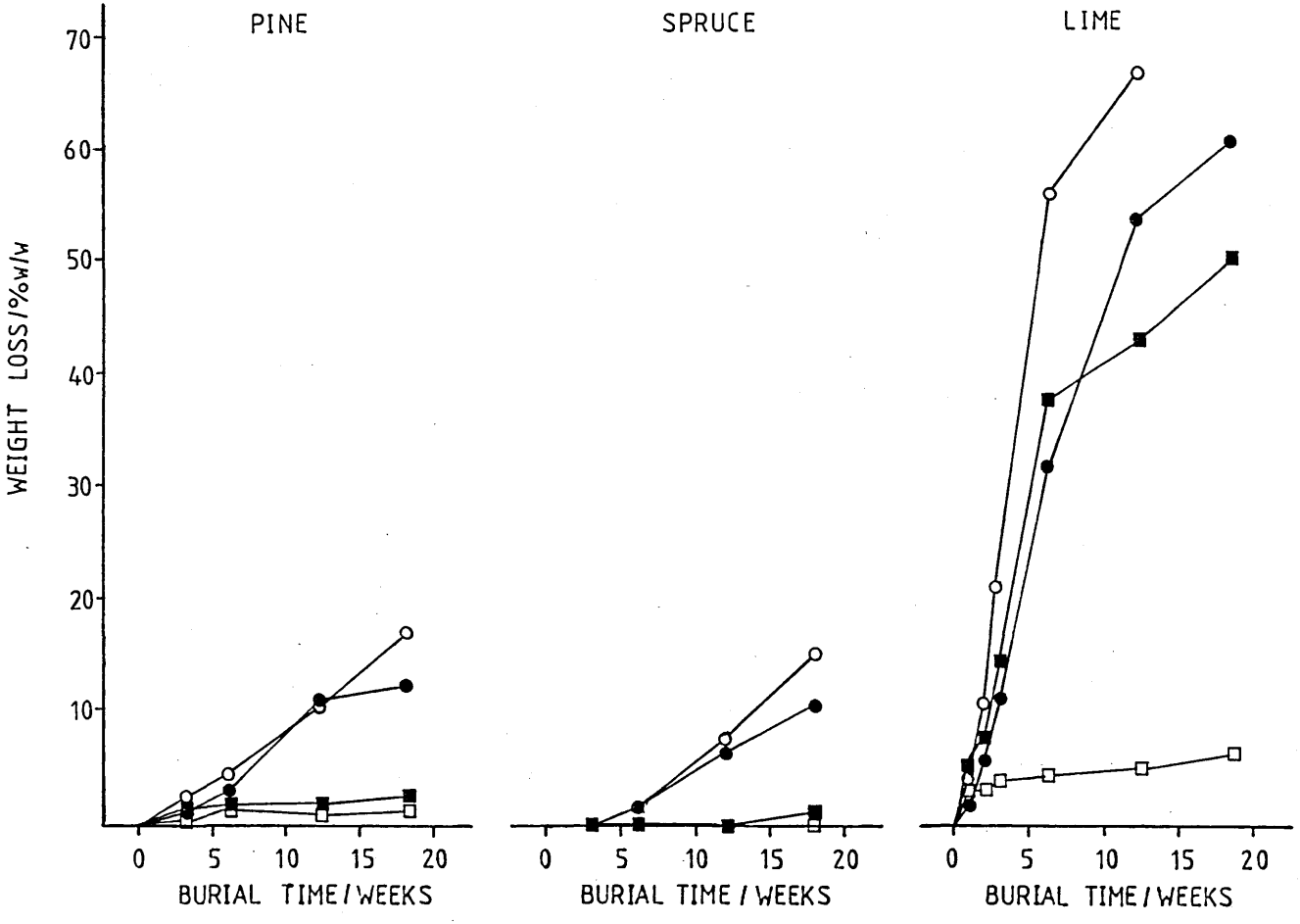


Figure 3.3.2.1 Average weight losses of wood blocks during the soil burial study. Average is based on 4 replicates.

● Untreated.
○ Ammonia-treated.
■ 0.07/0.14%w/v ACA.
□ 1.41%w/v ACA.

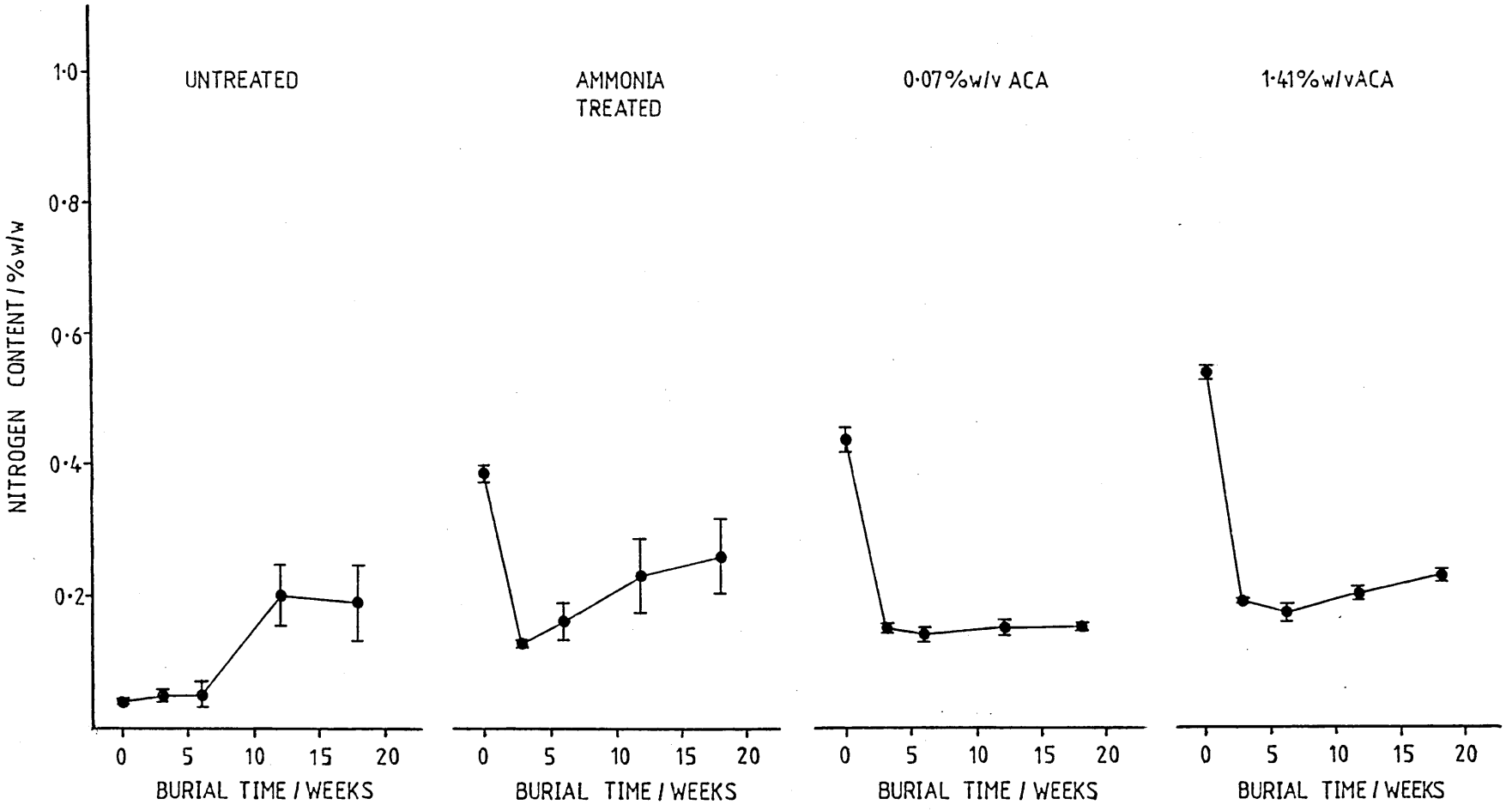


Figure 3.3.2.2 Mean nitrogen contents ± standard deviations of pine blocks during the soil burial study. Mean is based on 4 replicates.

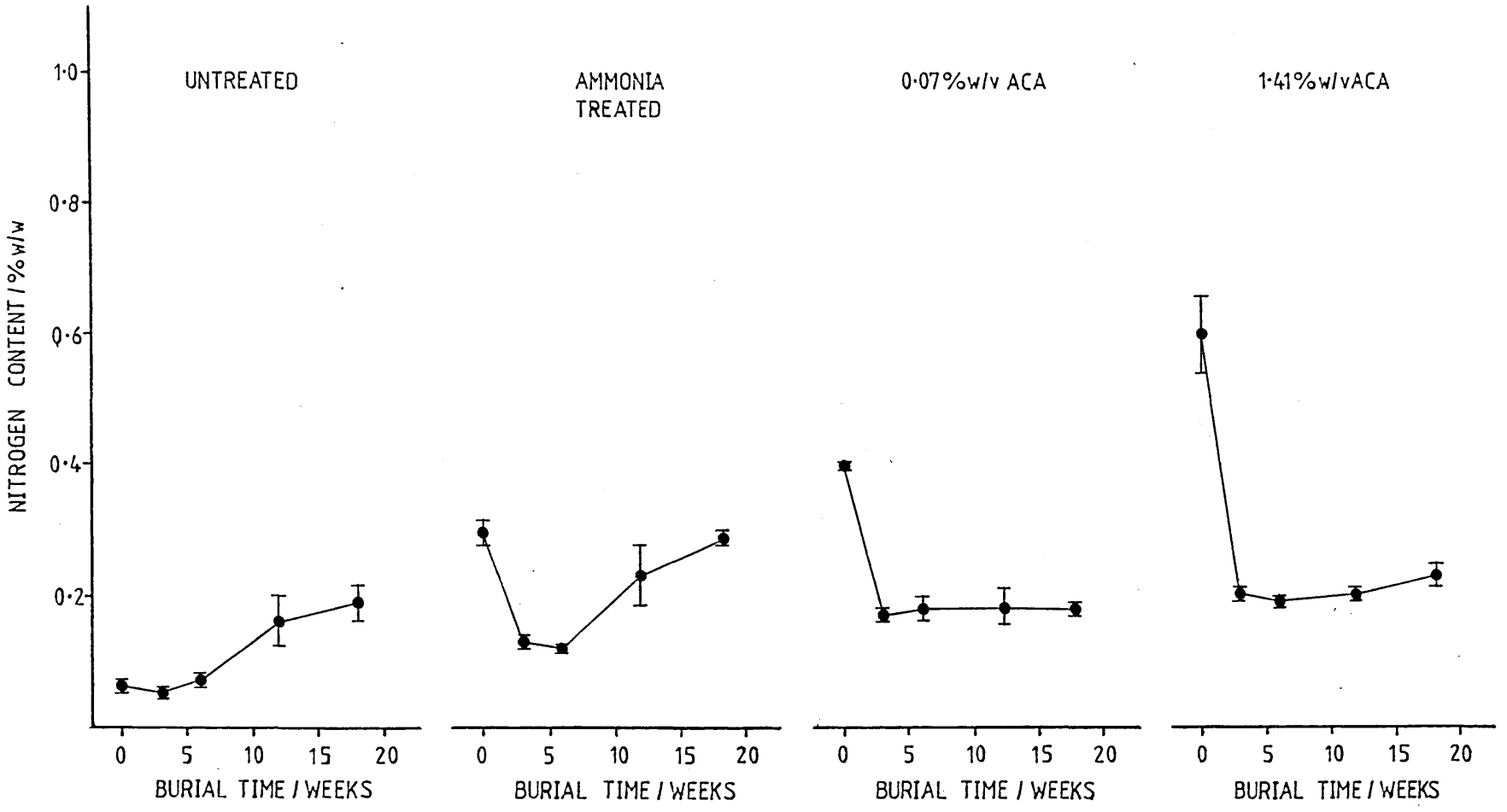


Figure 3.3.2.3 Mean nitrogen contents ± standard deviations of spruce blocks during the soil burial study. Mean is based on 4 replicates.

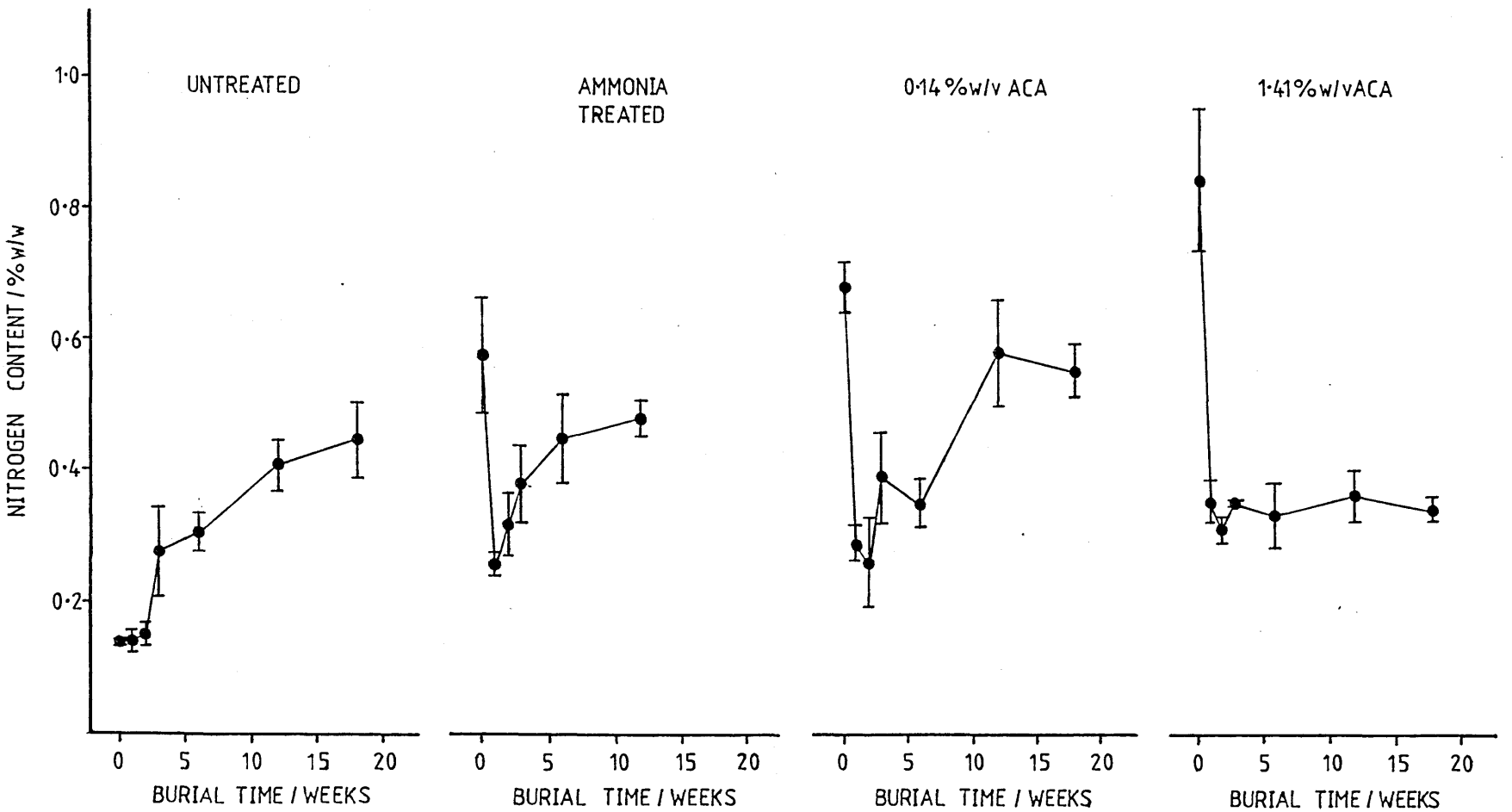


Figure 3.3.2.4 Mean nitrogen contents ± standard deviations of lime blocks during the soil burial study. Mean is based on 4 replicates.

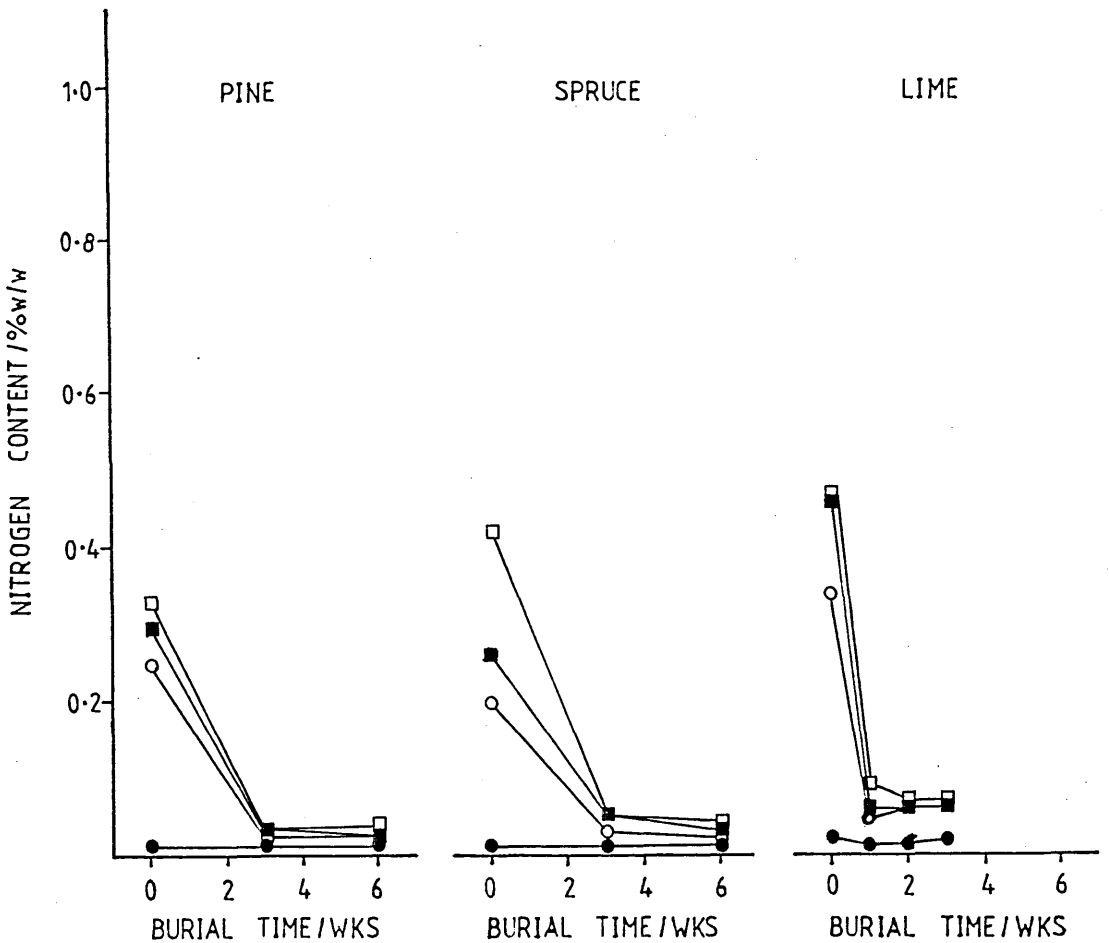


Figure 3.3.2.5 Average nitrogen as ammonia contents of wood blocks during the soil burial study. Average is based on a minimum of 4 replicates.

● Untreated.
○ Ammonia-treated.
■ 0.07/0.14%w/v ACA.
□ 1.41%w/v ACA.

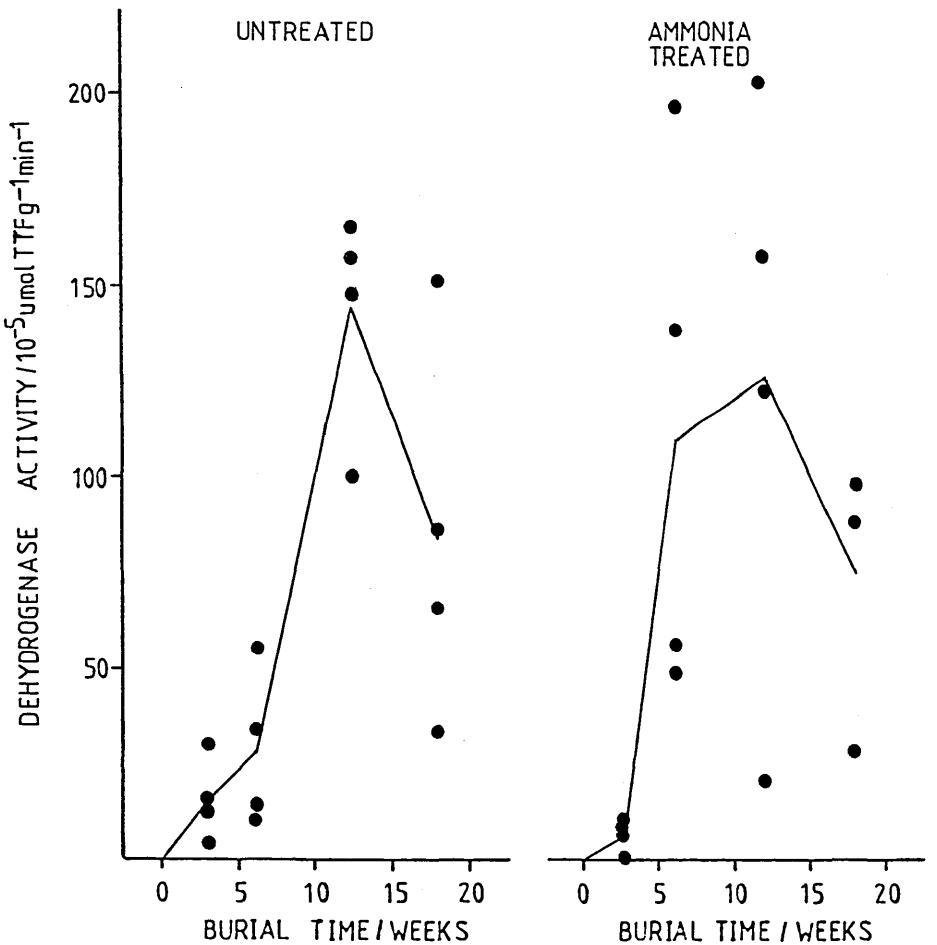


Figure 3.3.2.6 Dehydrogenase activity in the outer wood surface of pine blocks during the soil burial study. Individual results are shown and a curve is drawn through the average levels. Average is base on 4 replicates.

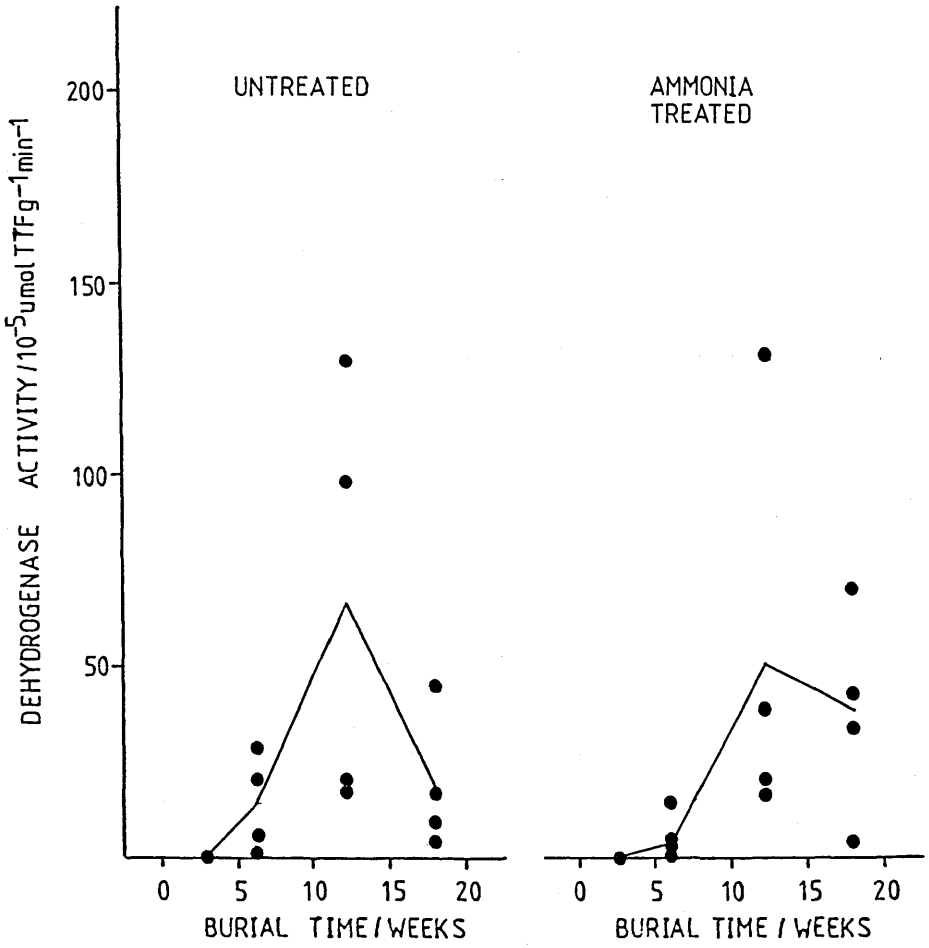


Figure 3.3.2.7 Dehydrogenase activity in the outer wood surface of spruce blocks during the soil burial study. Individual results are shown and a curve is drawn through the average levels. Average is base on 4 replicates.

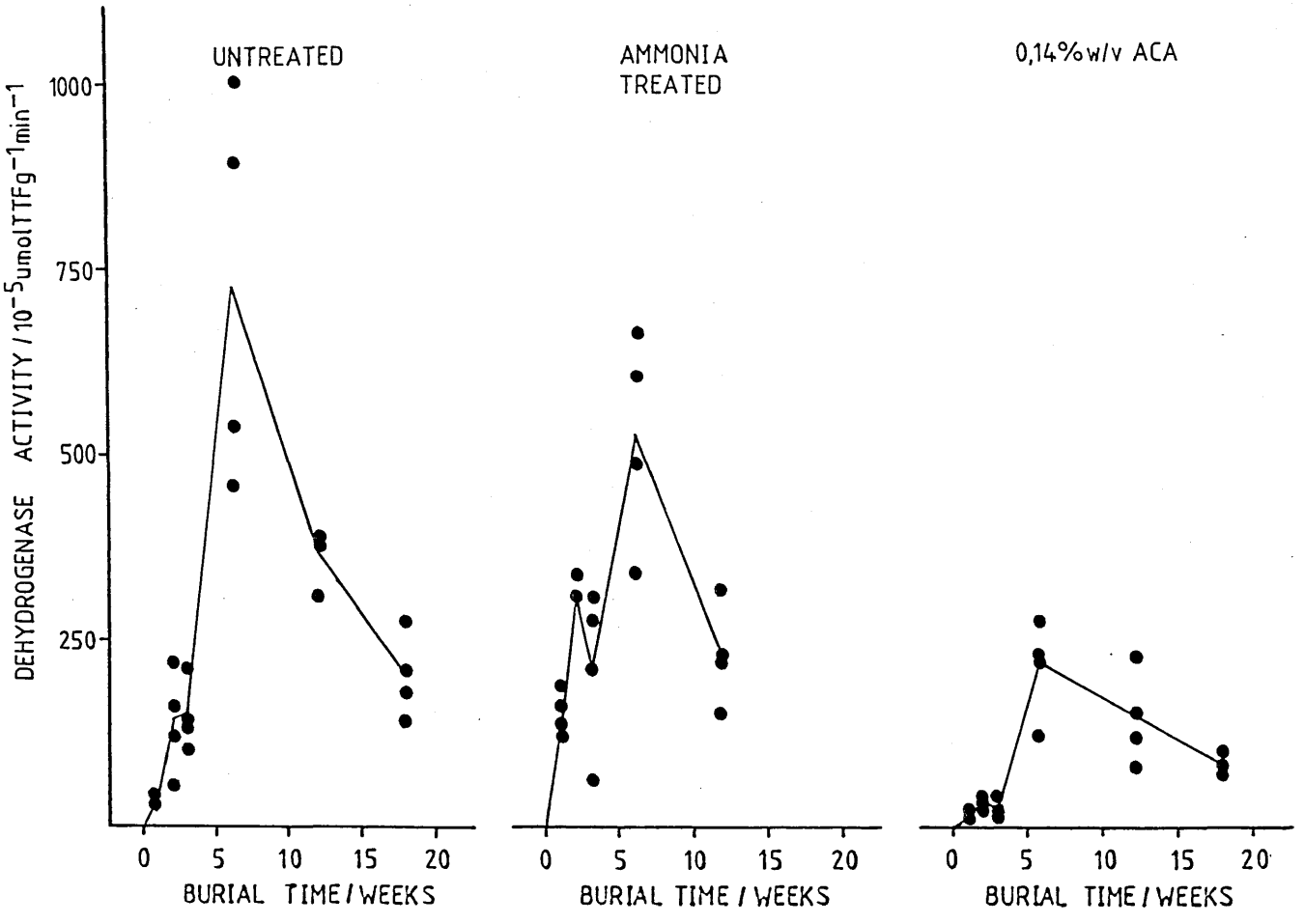


Figure 3.3.2.8 Dehydrogenase activity in the outer wood surface of lime blocks during the soil burial study. Individual results are shown and a curve is drawn through the average levels. Average is base on 4 replicates.

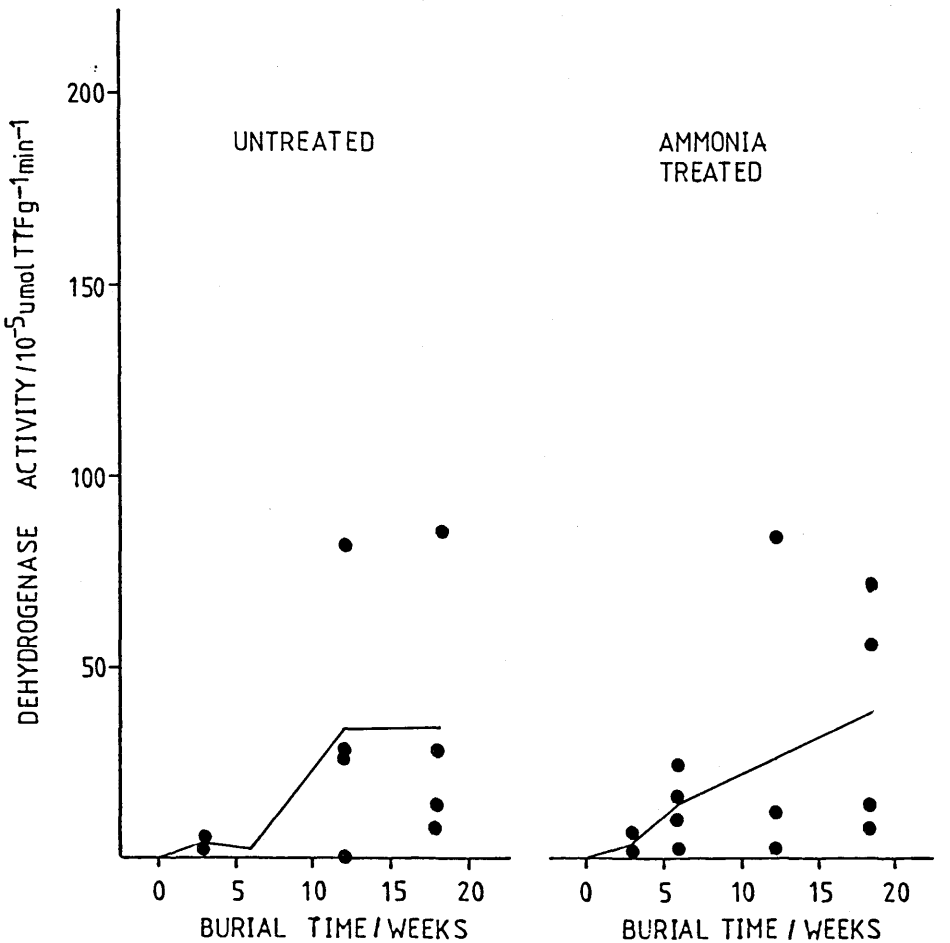


Figure 3.3.2.9 Dehydrogenase activity in the inner wood of pine blocks during the soil burial study. Individual results are shown and a curve is drawn through the average levels. Average is base on 4 replicates.

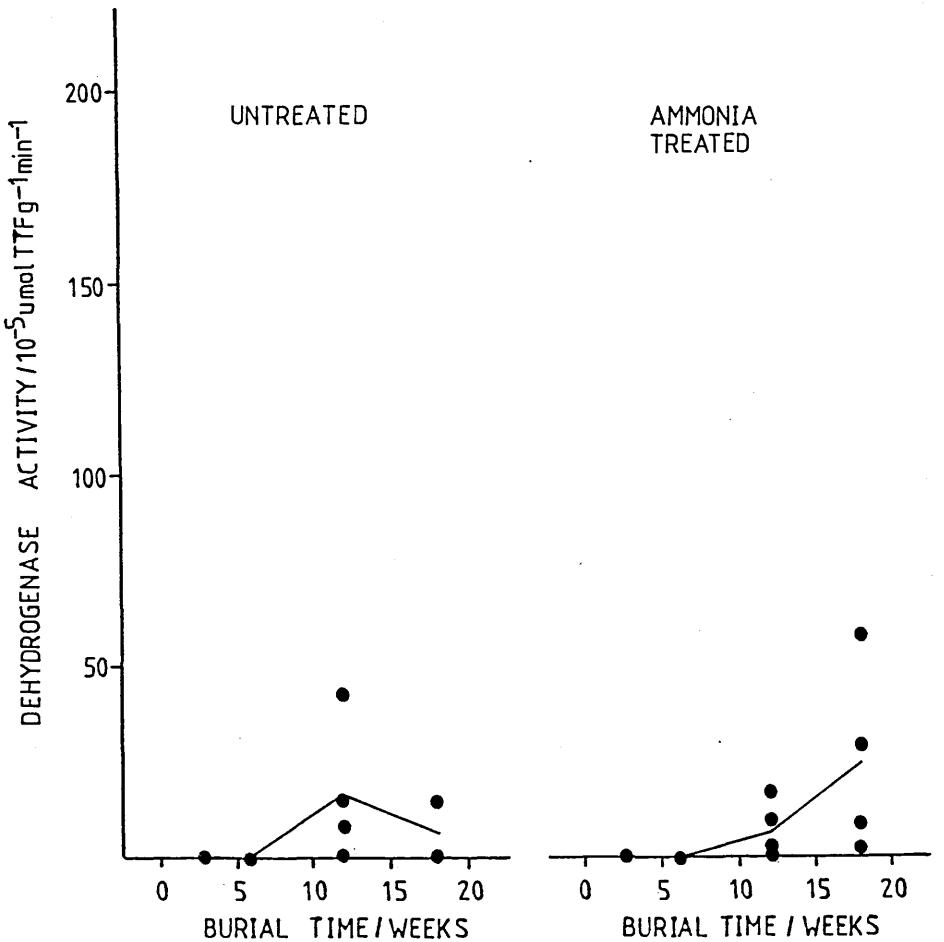


Figure 3.3.2.10 Dehydrogenase activity in the inner wood of spruce blocks during the soil burial study. Individual results are shown and a curve is drawn through the average levels. Average is base on 4 replicates.

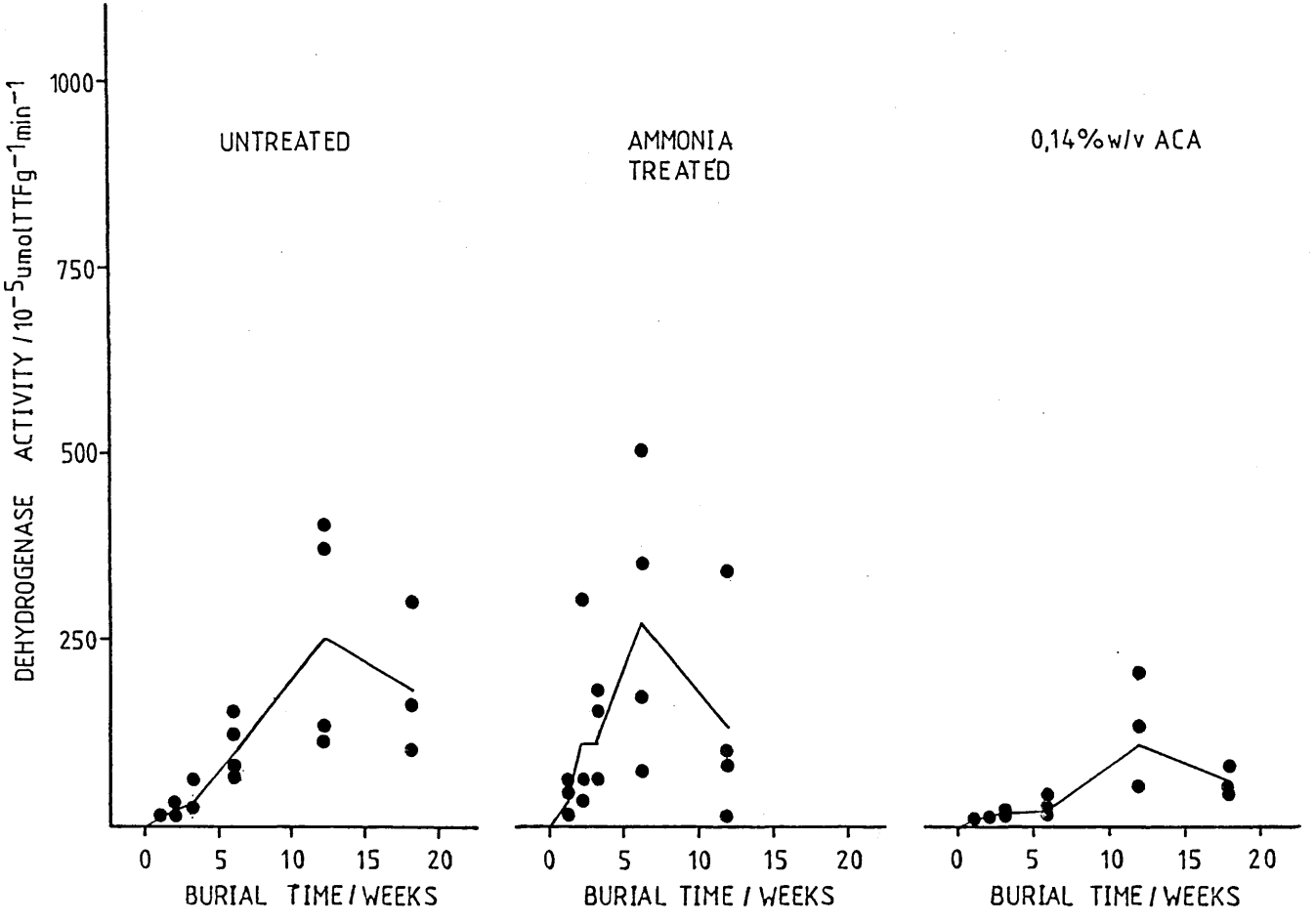


Figure 3.3.2.11 Dehydrogenase activity in the inner wood of lime blocks during the soil burial study. Individual results are shown and a curve is drawn through the average levels. Average is base on 4 replicates.

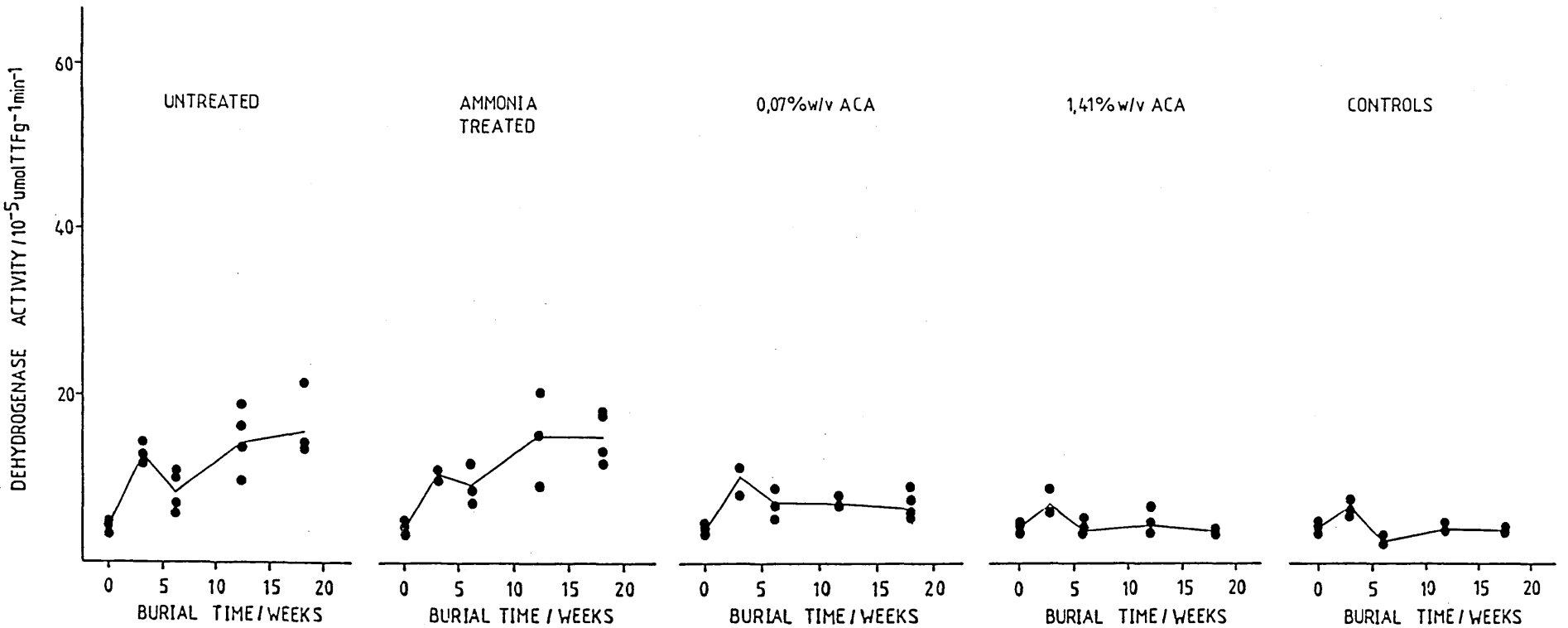


Figure 3.3.2.12 Dehydrogenase activity in soil adjacent to the pine blocks during the soil burial study. Individual results are shown and a curve is drawn through the average levels. Average is base on 4 replicates.

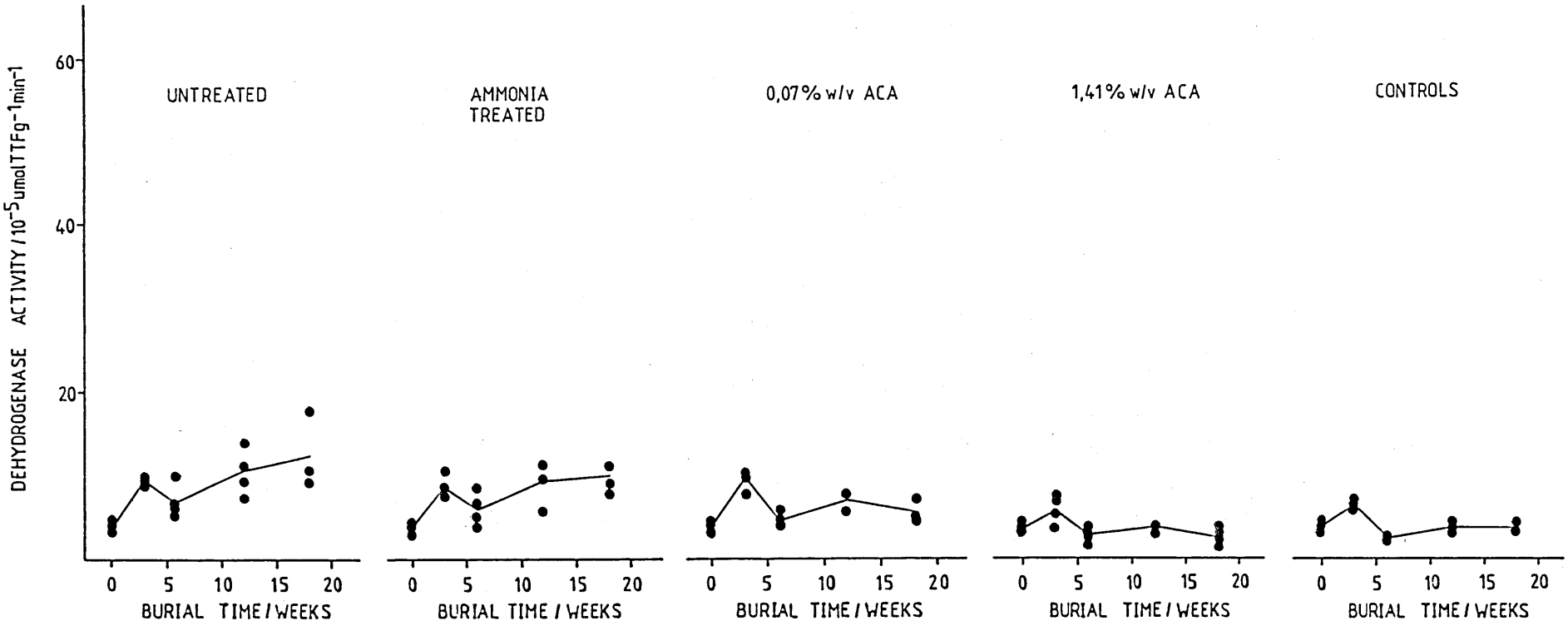


Figure 3.3.2.13 Dehydrogenase activity in soil adjacent to the spruce blocks during the soil burial study. Individual results are shown and a curve is drawn through the average levels. Average is base on 4 replicates.

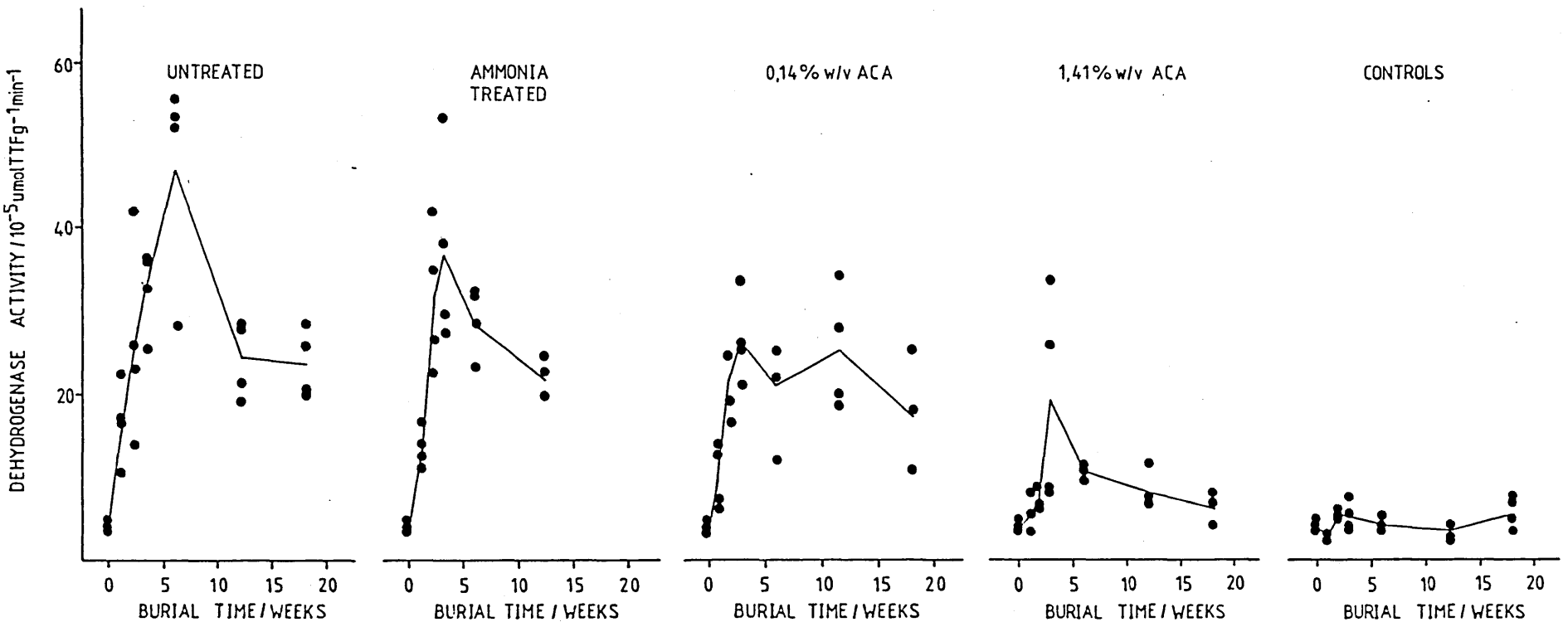


Figure 3.3.2.14 Dehydrogenase activity in soil adjacent to the lime blocks during the soil burial study. Individual results are shown and a curve is drawn through the average levels. Average is base on 4 replicates.

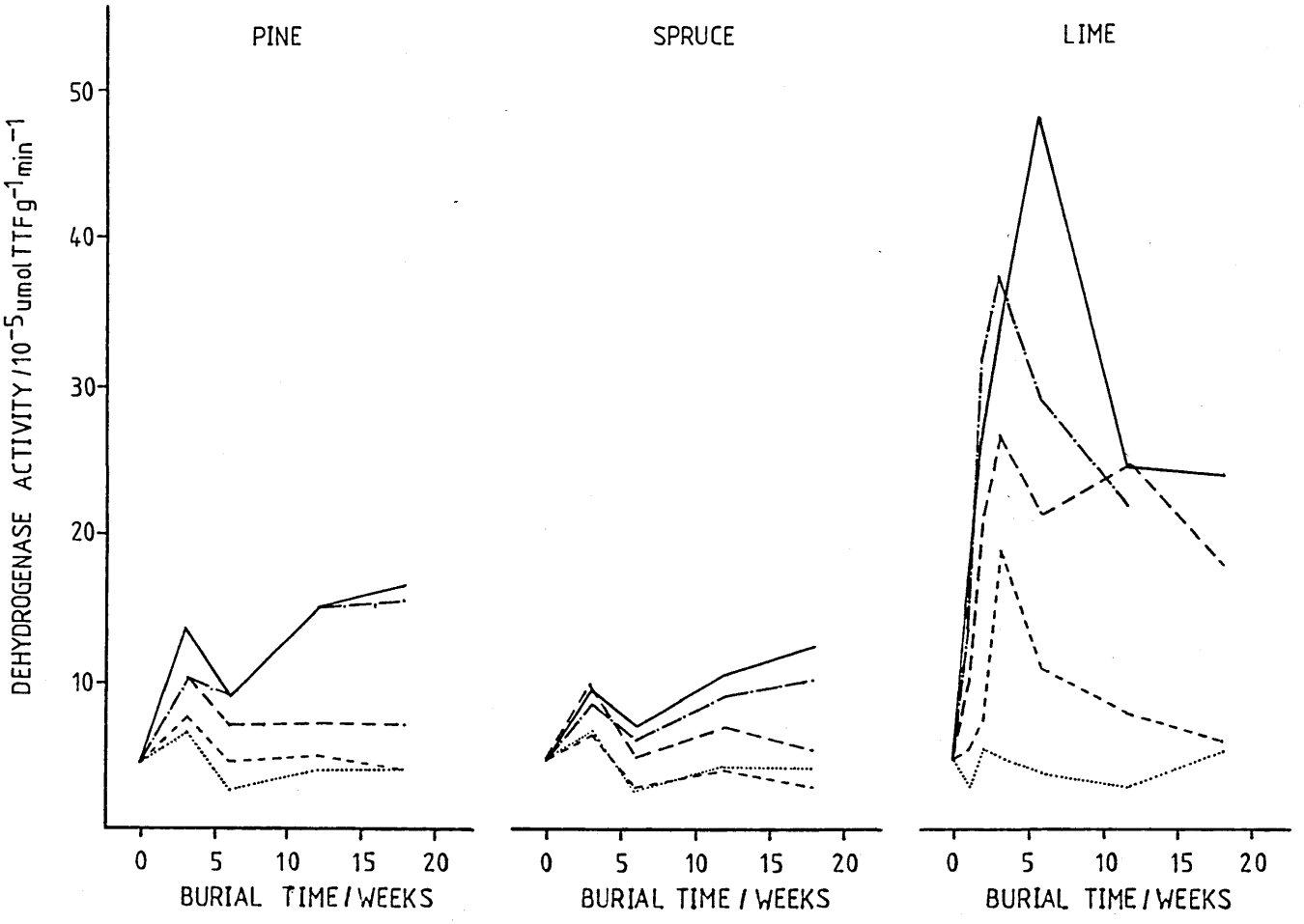


Figure 3.3.2.15 Average dehydrogenase activity in soil adjacent to the wood blocks during the soil burial study. Average is base on 4 replicates.

—— Untreated.
—·—· Ammonia-treated.
– – – 0.07/0.14%w/v ACA.
----- 1.41%w/v ACA.
····· Controls.

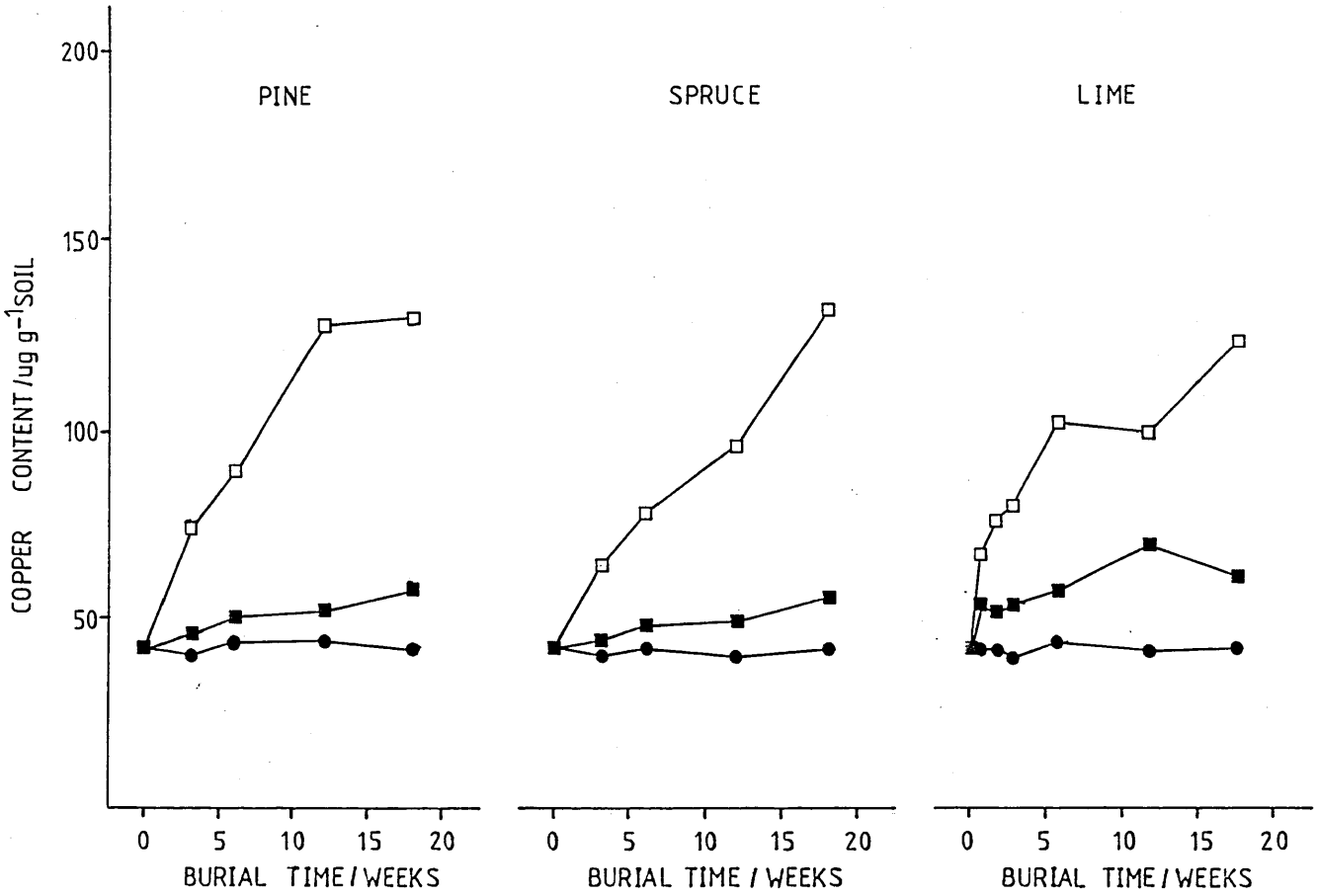


Figure 3.3.2.16 Average copper contents of soil adjacent to the wood blocks. Average is based on 4 replicates.

● Untreated.
■ 0.07/0.14%w/v ACA.
□ 1.41%w/v ACA.

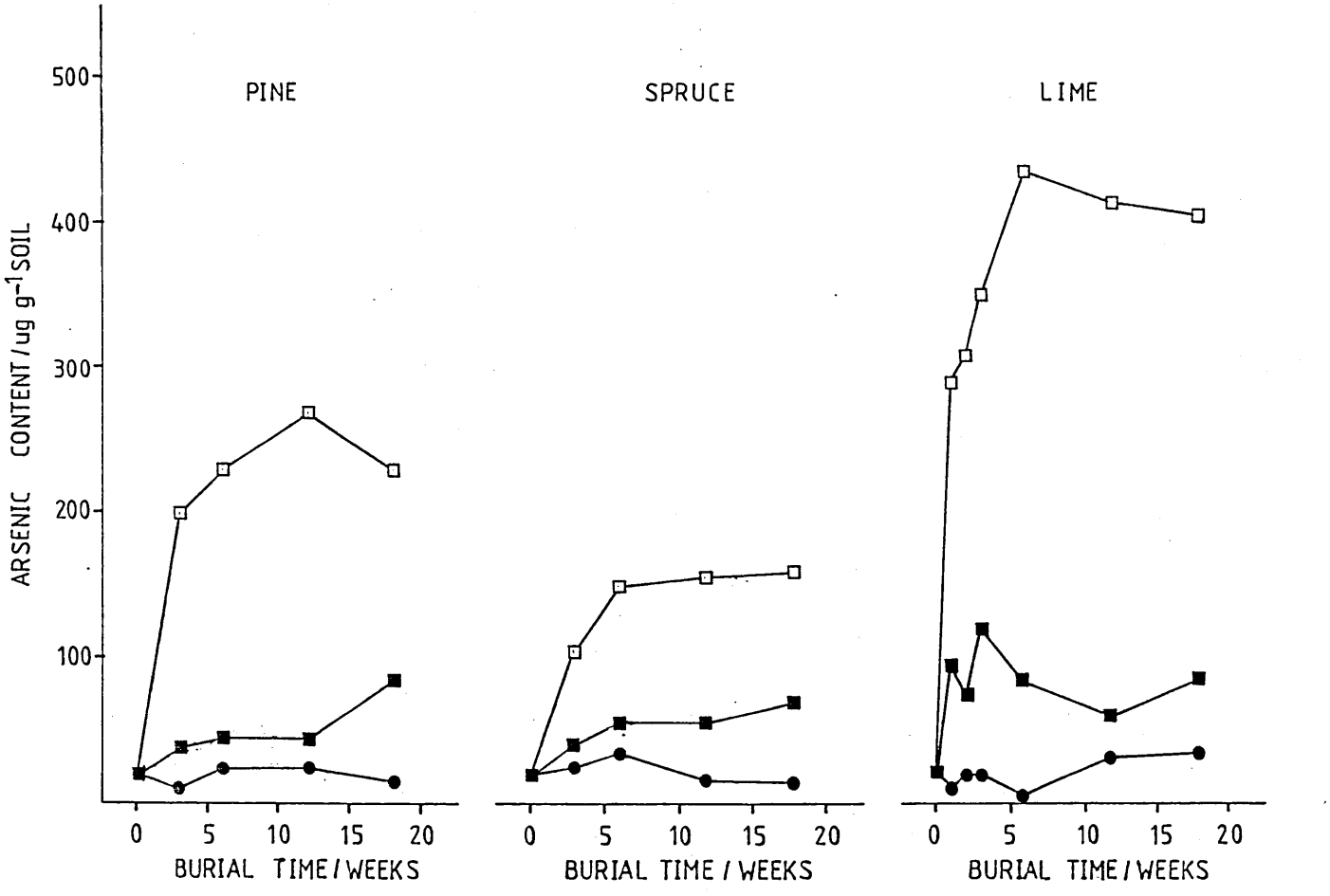


Figure 3.3.2.17 Average arsenic contents of soil adjacent to the wood blocks. Average is based on 4 replicates.

● Untreated.
■ 0.07/0.14%w/v ACA.
□ 1.41%w/v ACA.

3.3.2.12 Tables 3.3.2.1-3.3.2.34.

Table 3.3.2.1 Moisture contents of softwood blocks during the study.

Table 3.3.2.2 Moisture contents of lime blocks during the study.

Table 3.3.2.3 Results of statistical analysis to assess the effect of ammonia on the wood moisture content.

Table 3.3.2.4 Weight losses of softwood blocks during the study.

Table 3.3.2.5 Weight losses of lime blocks during the study.

Table 3.3.2.6 Results of statistical analysis to assess the effect of ammonia on wood weight loss.

Table 3.3.2.7 Estimated time of initiation of microbial decay.

Table 3.3.2.8 Estimated rate of decay.

Table 3.3.2.9 Nitrogen contents of softwood blocks during the study.

Table 3.3.2.10 Nitrogen contents of lime blocks during the study.

Table 3.3.2.11 Statistical comparison of nitrogen contents data for wood blocks during the study.

Table 3.3.2.12 Estimated nitrogen contents at time of initiation of microbial decay.

Table 3.3.2.13 Nitrogen as ammonia contents of softwood blocks during the study.

Table 3.3.2.14 Nitrogen as ammonia contents of lime blocks during the study.

Table 3.3.2.15 Assessment of the significance of the nitrogen as ammonia contents in relation to the nitrogen contents of the wood blocks. I. The decrease in the nitrogen contents of ammonia and ACA-treated blocks on soil burial.

Table 3.3.2.16 Assessment of the significance of the nitrogen as ammonia contents in relation to the nitrogen contents of the wood blocks. II. "Additional" nitrogen present in buried, ammonia and ACA-treated blocks.

Table 3.3.2.17 Copper contents, based on liquid uptake, of wood blocks.

Table 3.3.2.18 Arsenic contents, based on liquid uptake, of wood blocks.

Table 3.3.2.19 Copper contents of wood blocks from the study, based on analysis.

Table 3.3.2.1 Moisture contents of softwood blocks during the soil burial study. Mean results ± standard deviations are presented (mean is based on 4 replicates).

Wood species	Burial time (weeks)	Moisture content (%w/w) Untreated	Ammonia treated	0.07%w/v ACA	1.41%w/v ACA
Pine	3	36.2± 1.1	39.2± 2.1	40.0± 1.1	39.6± 2.1
	6	39.1± 2.2	45.6± 4.1	40.2± 2.2	40.2± 1.5
	12	52.0± 7.7	51.5± 5.4	39.0± 2.0	39.3± 1.7
	18	52.2+10.1	63.2+16.0	40.0+ 4.0	40.0+ 2.0
Spruce	3	38.9± 3.5	40.3± 1.5	40.1± 3.2	40.1± 2.4
	6	41.5± 4.5	41.4± 2.2	41.0± 2.0	39.1± 1.6
	12	44.5± 4.9	48.3± 8.0	39.9± 2.7	40.5± 2.3
	18	51.1+10.1	59.8+10.4	40.3+ 3.0	39.8+ 2.1

Table 3.3.2.2 Moisture contents of lime blocks during the soil burial study. Mean results ± standard deviations are presented (Mean is based on 4 replicates).

Wood species	Burial time (weeks)	Moisture content (%w/w) Untreated	Ammonia treated	0.14%w/v ACA	1.41%w/v ACA
Lime	1	42.9± 2.2	47.5± 0.7	46.8± 0.5	44.1± 1.3
	2	45.0± 4.5	52.6± 3.6	49.9± 0.9	43.8± 1.8
	3	55.2± 8.4	62.0± 7.5	55.9± 2.3	45.7± 0.9
	6	74.8± 6.4	97.8± 9.9	73.6± 9.3	45.7± 1.0
	12	114.6±28.7	112.1±14.8	89.7±17.2	46.5± 0.8
	18	118.3+18.0	M/N	103.0+25.5	47.5+ 2.1

Key. M/N measurement not carried out.

Table 3.3.2.3 Results of statistical analysis (two-way analysis of variance) to assess differences in moisture contents of untreated and ammonia-treated wood blocks during the soil burial study.

Wood species	Interaction	Factor Time	Factor Ammonia
Pine	NS	***	NS
Spruce	NS	***	NS
Lime	NS	***	*

Key. NS No significant difference.
* Significant difference: probability of difference arising by chance is < 5%.
*** Significant difference: probability of difference arising by chance is < 0.5%.

Table 3.3.2.4 Weight losses of softwood blocks during the soil burial study. Mean results ± standard deviations are presented (mean is based on 4 replicates).

Wood species	Burial time (weeks)	Weight loss (%w/w) Untreated	Ammonia treated	0.07%w/v ACA	1.41%w/v ACA
Pine	3	1.36±0.58	2.68±0.16	1.82±0.62	0.38±0.43
	6	3.45±0.31	4.69±0.58	1.97±0.41	1.40±0.21
	12	11.75±3.48	11.37±1.85	2.28±0.54	0.97±0.57
	18	13.55+3.54	18.97+6.59	2.65+0.48	1.48+0.34
Spruce	3	+0.11±0.18	1.77±1.68	0.39±0.52	0 ±0.53
	6	1.95±1.92	2.01±1.08	0.47±1.08	0.78±0.50
	12	6.74±0.48	7.70±4.10	0.08±1.25	+0.11±0.47
	18	11.39+3.51	15.83+3.31	1.14+0.95	+0.04+0.42

Table 3.3.2.5 Weight losses of lime blocks during the soil burial study. Mean results ± standard deviations are presented (mean is based on 4 replicates).

Wood species	Burial time (weeks)	Weight loss (%w/w) Untreated	Ammonia treated	0.14%w/v ACA	1.41%w/v ACA
Lime	1	2.29±0.74	4.60±0.41	4.95±0.43	3.75±0.43
	2	6.04±2.21	11.62±1.79	8.76±0.70	3.90±0.80
	3	11.62±4.05	22.65±2.28	15.65±3.24	4.12±0.44
	6	33.67±5.96	59.03±5.26	39.76±8.26	5.22±0.98
	12	56.71±8.68	70.48±4.50	45.23±6.10	5.59±0.61
	18	64.35+7.84	M/N	53.16+9.69	6.70+1.05

Key. M/N measurement not carried out.

Table 3.3.2.6 Results of statistical analysis (two-way analysis of variance) to assess differences in weight loss of untreated and ammonia-treated wood blocks in the soil burial study.

Wood species	Interaction	Factor Time	Factor Ammonia
Pine	NS	***	NS
Spruce	NS	***	***
Lime	***	***	***

Key. NS No significant difference.
*** Significant difference: probability of difference arising by chance is < 0.5%.

Table 3.3.2.7 Estimated time of initiation of microbial decay (weeks).

Wood species	Treatment			
	Untreated	Ammonia	0.07/0.14%	1.41%w/v ACA
Pine	4.80	3.20	<3%	<3%
Spruce	6.80	6.80	<3%	<3%
Lime	1.20	0.60	0.40	1.00 (*)

Key. <3% average weight loss did not exceed 3% throughout this soil burial study.
(*) weight loss exceeded 3%, but considered unlikely to be due to decay.

Table 3.3.2.8 Estimated rate of decay (%/week).

Wood species	Treatment			
	Untreated	Ammonia	0.07/0.14%	1.41%w/v ACA
Pine	0.80	1.28	N/A	N/A
Spruce	0.75	1.41	N/A	N/A
Lime	3.65	6.20	3.02	N/A

Key. N/A calculation not applicable.

Table 3.3.2.9 Nitrogen contents of softwood blocks during the soil burial study. Mean results ± standard deviations are presented (mean is based on 4 replicates).

Wood species	Burial time (weeks)	Nitrogen content (%w/w) Untreated	Ammonia treated	0.07%w/v ACA	1.41%w/v ACA
Pine	Unburied	0.044±0.003	0.389±0.014	0.440±0.022	0.537±0.010
	3	0.054±0.009	0.124±0.006	0.154±0.006	0.193±0.003
	6	0.054±0.021	0.156±0.032	0.139±0.010	0.174±0.016
	12	0.205±0.050	0.228±0.062	0.154±0.008	0.203±0.010
	18	0.189+0.059	0.256+0.059	0.149+0.006	0.190+0.011
Spruce	Unburied	0.059±0.012	0.296±0.017	0.398±0.006	0.595±0.056
	3	0.050±0.006	0.129±0.008	0.166±0.012	0.204±0.014
	6	0.071±0.015	0.125±0.005	0.178±0.018	0.187±0.013
	12	0.156±0.038	0.232±0.047	0.184±0.029	0.201±0.010
	18	0.190+0.029	0.286+0.015	0.185+0.009	0.227+0.023

Table 3.3.2.10 Nitrogen contents of lime blocks during the soil burial study. Mean results ± standard deviations are presented (mean is based on 4 replicates).

Wood species	Burial time (weeks)	Nitrogen content (%w/w) Untreated	Ammonia treated	0.14%w/v ACA	1.41%w/v ACA
Lime	Unburied	0.140±0.005	0.583±0.095	0.684±0.040	0.836±0.113
	1	0.136±0.024	0.264±0.017	0.290±0.029	0.348±0.027
	2	0.149±0.019	0.324±0.050	0.259±0.067	0.313±0.016
	3	0.284±0.074	0.379±0.062	0.391±0.070	0.349±0.005
	6	0.311±0.032	0.453±0.072	0.350±0.041	0.330±0.047
	12	0.411±0.039	0.476±0.032	0.583±0.081	0.361±0.038
	18	0.452+0.059	M/N	0.551+0.039	0.337+0.016

Key. M/N measurement not carried out.

Table 3.3.2.11 Statistical comparison (one-way analysis of variance) of nitrogen contents data for wood blocks during the soil burial study.

Wood Species	Untreated	Ammonia treated	0.07/0.14%w/v ACA-treated	1.41%w/v ACA-treated
Pine	***	**	NS	*
Spruce	***	***	NS	*
Lime	***	***	***	NS

Key. NS No significant change.
* Significant difference: probability of difference arising by chance is < 5%.
** Significant difference: probability of difference arising by chance is < 1%.
*** Significant difference: probability of difference arising by chance is < 0.5%.

Table 3.3.2.12 Estimated nitrogen contents at time of initiation of microbial decay (%w/w).

Wood species	Treatment			
	Untreated	Ammonia	0.07/0.14%	1.41%w/v ACA
Pine	0.054	0.124	N/A	N/A
Spruce	0.080	0.135	N/A	N/A
Lime	0.140	0.124	0.290	N/A

Key. N/A calculation not applicable.

Table 3.3.2.13 Nitrogen as ammonia contents of softwood blocks during the soil burial study. Mean results ± standard deviations are presented (mean is based on 4 replicates).

Wood species	Burial time (weeks)	Nitrogen as ammonia content (%w/w)			
		Untreated	Ammonia treated	0.07%w/v ACA	1.41%w/v ACA
Pine	Unburied	0.009±0.001	0.246±0.013	0.296±0.006	0.333±0.021
	3	0.009±0.007	0.023±0.006	0.034±0.011	0.032±0.007
	6	0.007+0.002	0.022+0.006	0.025+0.002	0.037+0.008
Spruce	Unburied	0.008±0.002	0.200±0.020	0.265±0.023	0.425±0.052
	3	0.008±0.002	0.034±0.003	0.047±0.011	0.053±0.010
	6	0.007+0.002	0.024+0.005	0.029+0.013	0.037+0.008

Table 3.3.2.14 Nitrogen as ammonia contents of lime blocks during the soil burial study. Mean results ± standard deviations are presented (mean is based on 4 replicates).

Wood species	Burial time (weeks)	Nitrogen as ammonia content (%w/w)			
		Untreated	Ammonia treated	0.14%w/v ACA	1.41%w/v ACA
Lime	Unburied	0.016±0.004	0.344±0.077	0.462±0.022	0.473±0.034
	1	0.009±0.002	0.053±0.005	0.059±0.009	0.088±0.012
	2	0.009±0.003	0.059±0.013	0.064±0.004	0.073±0.003
	3	0.017+0.005	0.060+0.008	0.061+0.011	0.074+0.007

Table 3.3.2.15 Assessment of the significance of the nitrogen as ammonia contents in relation to the nitrogen contents of the wood blocks. I. The decrease in the nitrogen contents of ammonia and ACA-treated blocks on soil burial. The nitrogen as ammonia data was derived from tables 3.3.2.13 and 3.3.2.14, while the nitrogen data was taken from tables 3.3.2.9 and 3.3.2.10.

Wood species	Wood treatment	Decrease in nitrogen on burial (%w/w)		(B) as a percentage of (A)
		Total nitrogen (A)	Nitrogen as ammonia (B)	
Pine	ammonia	0.265	0.223	84
	0.07%w/v ACA	0.286	0.262	92
	1.41%w/v ACA	0.344	0.301	87
Spruce	ammonia	0.167	0.166	99
	0.07%w/v ACA	0.232	0.218	94
	1.41%w/v ACA	0.391	0.372	95
Lime	ammonia	0.319	0.291	91
	0.07%w/v ACA	0.394	0.403	102
	1.41%w/v ACA	0.488	0.385	79

Table 3.3.2.16 Assessment of the significance of the nitrogen as ammonia contents in relation to the nitrogen contents of the wood blocks. II. "Additional" nitrogen present in buried, ammonia and ACA-treated blocks. The nitrogen as ammonia data was derived from tables 3.3.2.13 and 3.3.2.14, while the nitrogen data was taken from tables 3.3.2.9 and 3.3.2.10.

Wood species	Wood treatment	Nitrogen in buried blocks (%w/w)		(D) as a percentage of (C)
		"Additional" (C)	Nitrogen as ammonia (D)	
Pine	ammonia	0.080	0.023	29
	0.07%w/v ACA	0.110	0.034	31
	1.41%w/v ACA	0.149	0.032	21
Spruce	ammonia	0.070	0.034	49
	0.07%w/v ACA	0.107	0.047	44
	1.41%w/v ACA	0.145	0.053	36
Lime	ammonia	0.124	0.053	43
	0.07%w/v ACA	0.150	0.059	39
	1.41%w/v ACA	0.208	0.088	42

Table 3.3.2.17 Copper contents, based on liquid uptake, of unburied wood blocks, and of comparable wood blocks intended for soil burial. Mean results ± standard deviations are presented (mean is based on 4 replicates).

Wood species	Burial time (weeks)	Copper content (%w/w) 0.07/0.14%w/v ACA	1.41%w/v ACA
Pine	Unburied	0.031±0.002	0.724±0.004
	3	0.032±0.001	0.720±0.038
	6	0.032±0.001	0.759±0.066
	12	0.031±0.002	0.751±0.041
	18	0.032+0.003	0.740+0.045
Spruce	Unburied	0.053±0.002	1.201±0.067
	3	0.052±0.002	1.151±0.144
	6	0.050±0.003	1.079±0.051
	12	0.053±0.004	1.177±0.117
	18	0.053+0.005	1.164+0.126
Lime	Unburied	0.060±0.001	0.621±0.017
	1	0.060±0.002	0.634±0.011
	2	0.059±0.001	0.614±0.016
	3	0.061±0.002	0.644±0.015
	6	0.062±0.002	0.615±0.028
	12	0.062±0.003	0.624±0.013
	18	0.062+0.002	0.609+0.011

Table 3.3.2.18 Arsenic contents, based on liquid uptake, of unburied wood blocks, and of comparable wood blocks intended for soil burial. Mean results ± standard deviations are presented (mean is based on 4 replicates).

Wood species	Burial time (weeks)	Arsenic content (%w/w) 0.07/0.14%w/v ACA	1.41%w/v ACA
Pine	Unburied	0.031±0.002	0.524±0.003
	3	0.032±0.001	0.521±0.027
	6	0.032±0.001	0.549±0.048
	12	0.031±0.002	0.543±0.030
	18	0.032+0.003	0.535+0.033
Spruce	Unburied	0.054±0.002	0.870±0.049
	3	0.054±0.002	0.834±0.104
	6	0.051±0.004	0.781±0.037
	12	0.054±0.004	0.852±0.085
	18	0.054+0.006	0.843+0.091
Lime	Unburied	0.052±0.001	0.450±0.012
	1	0.052±0.002	0.459±0.008
	2	0.052±0.001	0.444±0.012
	3	0.053±0.002	0.466±0.011
	6	0.054±0.002	0.445±0.021
	12	0.054±0.002	0.452±0.009
	18	0.055+0.002	0.441+0.008

Table 3.3.2.19 Copper contents of wood blocks from the soil burial study. Mean results ± standard deviations are presented (mean is based on 4 replicates).

Wood species	Burial time (weeks)	Copper content (%w/w) Untreated	0.07/0.14% ACA	1.41% ACA
Pine	Unburied	0.006±0.001	0.070±0.015	0.667±0.042
	18	0.008±0.001	0.055±0.003	0.609±0.058
Spruce	Unburied	0.008±0.002	0.095±0.010	1.207±0.134
	18	0.010±0.003	0.084±0.012	1.035±0.099
Lime	Unburied	0.005±0.001	0.099±0.011	0.577±0.109
	18	0.004±0.001	0.076±0.007	0.518±0.039

Table 3.3.2.20 Percentage loss of copper from wood blocks after 18 weeks of soil burial determined by the comparison method. Results of a T-test carried out on corresponding unburied and buried blocks are also presented.

Wood species	Untreated Loss	T-test	0.7/0.14% ACA Loss	T-test	1.41% ACA Loss	T-test
Pine	Inc	NS	21.4	**	8.7	NS
Spruce	Inc	NS	11.6	NS	14.2	NS
Lime	20.0	NS	23.2	*	10.2	NS

Key Inc Mean metal content of leached blocks is greater than that of unleached blocks.
NS No significant difference.
* Significant difference: probability of the difference arising by chance is < 5%.
** Significant difference: probability of the difference arising by chance is < 1%.

Table 3.3.2.21 Arsenic contents of wood blocks from the soil burial study. Mean results ± standard deviations are presented (mean is based on 4 replicates).

Wood species	Burial time (weeks)	Arsenic content (%w/w) Untreated	0.07/0.14% ACA	1.41% ACA
Pine	Unburied	0.005±0.007	0.029±0.017	0.413±0.022
	18	0.010±0.011	0.019±0.007	0.407±0.085
Spruce	Unburied	0.033±0.019	0.061±0.037	1.011±0.240
	18	0.014±0.011	0.022±0.022	0.638±0.110
Lime	Unburied	0.010±0.006	0.041±0.025	0.352±0.152
	18	0.004±0.006	0.005±0.004	0.142±0.077

Table 3.3.2.22 Percentage loss of arsenic from wood blocks after 18 weeks of soil burial determined by the comparison method. Results of a T-test carried out on corresponding unburied and buried blocks are also presented.

Wood species	Untreated Loss	T-test	0.7/0.14% ACA Loss	T-test	1.41% ACA Loss	T-test
Pine	Inc	NS	34.5	NS	1.4	ℱ
Spruce	57.6	NS	63.9	NS	36.9	*
Lime	60.0	NS	87.8	ℱ	59.6	*

Key Inc Mean metal content of leached blocks is greater than that of unleached blocks.
NS No significant difference.
ℱ F-test indicates that a T-test cannot be justified.
* Significant difference: probability of the difference arising by chance is < 5%.

Table 3.3.2.23 Dehydrogenase activity in the outer wood surface of softwood blocks during the soil burial study. Mean results ± standard deviations are presented (mean is based on 4 replicates).

Wood species	Burial time (weeks)	Dehydrogenase activity ($*10^{-5}$ umol TTF g^{-1} min^{-1})			
		Untreated	Ammonia treated	0.07%w/v ACA	1.41%w/v ACA
Pine	3	15.9±11.0	5.9± 4.6	1.5± 1.8	N/R
	6	28.5±21.0	110.6±71.4	N/R	N/R
	12	143.4±29.6	125.4±78.1	0.4± 0.7	N/R
	18	84.3±49.7	74.9±32.3	1.5± 3.0	N/R
Spruce	3	N/R	N/R	N/R	N/R
	6	13.7±13.3	4.9± 5.9	N/R	N/R
	12	66.0±56.7	50.9±54.2	N/R	N/R
	18	18.2±17.4	37.5±27.5	N/R	N/R

Table 3.3.2.24 Dehydrogenase activity in the outer wood surface of lime blocks during the soil burial study. Mean results ± standard deviations are presented (mean is based on 4 replicates).

Wood species	Burial time (weeks)	Dehydrogenase activity ($*10^{-5}$ umol TTF g^{-1} min^{-1})			
		Untreated	Ammonia treated	0.14%w/v ACA	1.41%w/v ACA
Lime	1	35.2± 3.2	155.0±31.3	15.0± 3.3	N/R
	2	138.0±73.7	311.0±17.2	26.5± 8.5	N/R
	3	146.0±48.4	215.0±112.0	24.1±16.7	N/R
	6	725.9±270.0	526.5±147.4	217.5±70.5	N/R
	12	369.2±39.8	230.7±69.9	146.2±61.4	N/R
	18	203.9±57.1	M/N	79.8±15.5	N/R

Key. M/N measurement not carried out.
N/R no reading obtained.

Table 3.3.2.25 Dehydrogenase activity in the inner wood of softwood blocks during the soil burial study. Mean results ± standard deviations are presented (mean is based on 4 replicates).

Wood species	Burial time (weeks)	Dehydrogenase activity ($*10^{-5}$ umol TTF g^{-1} min^{-1})			
		Untreated	Ammonia treated	0.07%w/v ACA	1.41%w/v ACA
Pine	3	4.9± 1.3	5.0± 2.6	0.6±1.3	N/R
	6	2.1± 0.4	13.2± 9.4	N/R	N/R
	12	34.3±34.7	25.4±39.3	N/R	N/R
	18	34.0±35.2	37.3±30.9	N/R	N/R
Spruce	3	N/R	N/R	N/R	N/R
	6	N/R	N/R	N/R	N/R
	12	15.8±18.0	6.7± 7.2	N/R	N/R
	18	6.7± 7.6	24.0±25.4	N/R	N/R

Table 3.3.2.26 Dehydrogenase activity in the inner wood of lime blocks during the soil burial study. Mean results ± standard deviations are presented (mean is based on 4 replicates).

Wood species	Burial time (weeks)	Dehydrogenase activity ($*10^{-5}$ umol TTF g^{-1} min^{-1})			
		Untreated	Ammonia treated	0.14%w/v ACA	1.41%w/v ACA
Lime	1	13.0± 5.0	34.3±18.1	5.5± 2.1	N/R
	2	18.6± 7.2	113.0±127.0	8.6± 2.8	N/R
	3	30.9±17.6	113.0± 62.9	15.3± 5.8	N/R
	6	104.1±42.5	272.9±191.2	21.6± 9.7	N/R
	12	252.0±153.1	130.4±143.0	105.7±71.8	N/R
	18	179.5±84.95	M/N	62.2±20.3	N/R

Key. M/N measurement not carried out.
N/R no reading obtained.

Table 3.3.2.27 Dehydrogenase activity in soil adjacent to softwood blocks during the soil burial study, and in soil at a distance from the wood blocks. Mean results ± standard deviations are presented (mean is based on 4 replicates).

Wood species	Burial time (weeks)	Dehydrogenase activity ($*10^{-5}$ umol TTF g^{-1} min^{-1})				
		Untreated	Ammonia treated	0.07%w/v ACA	1.41%w/v ACA	Soil at a distance
Pine	3	13.3±1.2	10.6±0.9	10.5±1.7	7.3±1.3	6.4±0.9
	6	8.8±2.5	9.0±2.3	7.1±1.5	4.3±0.6	2.6±0.3
	12	15.0±4.0	15.1±4.5	7.1±0.7	4.8±1.2	4.0±0.5
	18	16.3±4.0	15.6±3.1	6.9±1.6	3.8±0.3	4.1±0.4
Spruce	3	9.3±0.6	8.6±1.4	9.8±1.3	6.3±1.8	6.4±0.9
	6	7.0±2.0	6.1±1.9	4.9±0.7	3.0±1.1	2.6±0.3
	12	10.5±2.7	9.2±2.5	7.1±1.1	3.8±0.6	4.0±0.5
	18	12.3±4.1	10.0±1.7	5.6±1.0	2.8±1.0	4.1±0.4

Table 3.3.2.28 Dehydrogenase activity in soil adjacent to lime blocks during the a soil burial study, and in soil at a distance from the wood blocks. Mean results ± standard deviations are presented (mean is based on 4 replicates).

Wood species	Burial time (weeks)	Dehydrogenase activity ($*10^{-5}$ umol TTF g^{-1} min^{-1})				
		Untreated	Ammonia treated	0.14%w/v ACA	1.41%w/v ACA	Soil at a distance
Lime	1	15.1±6.2	13.4±2.7	10.0±3.8	5.4±2.0	2.8±0.4
	2	26.6±11.7	31.8±8.8	21.3±3.9	7.6±1.5	5.6±0.8
	3	33.4±5.1	37.5±12.4	26.8±5.1	19.2±12.9	5.0±1.7
	6	48.3±13.2	29.0±4.3	21.3±6.5	10.8±1.0	4.0±0.7
	12	24.4±4.8	21.8±2.1	25.5±7.6	8.0±2.2	3.2±0.9
	18	23.8±4.2	M/N	17.9±6.0	6.1±1.6	5.3±1.9

Key. M/N measurement not carried out.
N/R no reading obtained.

Table 3.3.2.29 Copper contents of soil adjacent to softwood blocks during the soil burial study. Mean results ± standard deviations are presented (mean is based on 4 replicates).

Wood species	Burial time (weeks)	Copper content (ug/g[dry weight]) Untreated	0.07%w/v ACA	1.41%w/v ACA
Pine	3	39.0±2.6	45.6±0.6	73.7±5.6
	6	43.2±1.1	50.6±4.8	89.1±9.1
	12	44.3±2.4	52.4±5.6	127.8±12.9
	18	41.7+1.0	57.6+3.9	129.4+25.5
Spruce	3	40.5±1.1	44.8±1.2	63.6±5.7
	6	41.8±0.9	48.8±4.4	77.2±4.0
	12	40.7±2.7	49.1±4.7	95.4±11.6
	18	43.9+2.1	55.3+4.1	132.8+5.4

Table 3.3.2.30 Copper contents of soil adjacent to lime blocks during the soil burial study. Mean results ± standard deviations are presented (mean is based on 4 replicates).

Wood species	Burial time (weeks)	Copper content (ug/g[dry weight]) Untreated	0.14%w/v ACA	1.41%w/v ACA
Lime	1	41.5±1.3	53.7±2.5	68.9±3.5
	2	42.4±1.6	51.7±1.7	75.2±6.7
	3	39.8±0.9	54.3±6.8	79.5±6.4
	6	43.5±4.3	57.3±8.7	102.1±10.6
	12	41.7±3.1	69.4±8.8	100.9±11.8
	18	42.9+2.1	61.5+4.5	124.7+35.2

Key. M/N measurement not carried out.
N/R no reading obtained.

Table 3.3.2.31 Arsenic contents of soil adjacent to softwood blocks during the soil burial study. Mean results ± standard deviations are presented (mean is based on 4 replicates).

Wood species	Burial time (weeks)	Arsenic content (ug/g[dry weight]) Untreated	0.07%w/v ACA	1.41%w/v ACA
Pine	3	7.6±6.0	39.3±17.2	198.3±41.4
	6	24.1±18.1	42.8±32.6	229.4±61.0
	12	25.2±40.6	44.6±4.5	268.4±54.5
	18	15.2+14.4	84.0+54.3	231.6+38.9
Spruce	3	22.9±6.5	38.6±13.5	103.7±15.9
	6	34.0±6.5	57.1±15.4	151.0±23.9
	12	16.2±24.1	54.5±23.8	157.5±24.0
	18	13.5+16.7	71.3+44.3	160.5+22.6

Table 3.3.2.32 Arsenic contents soil adjacent to lime blocks during the soil burial study. Mean results ± standard deviations are presented (mean is based on 4 replicates).

Wood species	Burial time (weeks)	Arsenic content (ug/g[dry weight]) Untreated	0.14%w/v ACA	1.41%w/v ACA
Lime	1	10.0±14.6	97.0±20.3	291.6±45.2
	2	20.9±16.8	74.0±22.9	311.2±47.2
	3	19.7±12.7	122.2±22.7	351.0±67.5
	6	6.2±7.7	85.6±41.7	433.8±35.0
	12	31.7±56.8	59.5±42.4	414.9±52.7
	18	35.0+23.4	83.3+27.7	403.3+73.0

Key. M/N measurement not carried out.
N/R no reading obtained.

Table 3.3.2.33 Results of statistical analyses (one-way analysis of variance) to investigate possible changes in preservative metal concentrations (ug g soil) of soil collected from around untreated and ACA-treated wood blocks during the soil burial study.

Wood species	Metal	One-way analysis of variance Untreated	0.07/0.14%w/v ACA	1.41%w/v ACA
Pine	copper	**	***	***
	arsenic	NS	NS	***
Spruce	copper	NS	***	***
	arsenic	NS	NS	***
Lime	copper	NS	***	***
	arsenic	NS	***	***

Key. NS No significant difference.
** Significant difference: probability of the difference arising by chance is < 1%.
** Significant difference: probability of the difference arising by chance is < 0.5%.

Table 3.3.2.34 Results of statistical analysis (two-way analysis of variance) to assess differences in preservative metal concentrations of soil collected from around untreated, and either 0.07%w/v ACA-treated blocks (pine and spruce) or 0.14%w/v ACA-treated blocks (lime) with time.

Wood species	Metal	Interaction	Factor Time	Treatment
Pine	Copper	***	***	***
	Arsenic	NS	NS	***
Spruce	Copper	***	***	***
	Arsenic	NS	NS	***
Lime	Copper	***	***	***
	Arsenic	*	*	***

Key. As table 3.3.2.31.

CHAPTER 4

DISCUSSION

DISCUSSION

4.1 Introduction.

Since a number of the parameters measured in in this project were common to all three experimental programmes the results are discussed under the following headings:

4.2 Moisture contents of buried, untreated and preservative treated wood blocks.

4.3 Weight loss of the buried wood blocks.

4.4 Changes in the nitrogen contents of wood blocks during cold water leaching and soil burial.

4.5 Preservative metal losses from CCA and ACA-treated wood blocks and their accumulation in adjacent soil.

4.6 Dehydrogenase activity in the outer and inner wood of buried wood blocks.

4.7 Dehydrogenase activity in soil adjacent to buried wood blocks.

4.2 Moisture contents of buried, untreated and preservative treated wood blocks.

The moisture contents of all buried wood blocks were in excess of the fibre saturation point (24-30%w/w, Wilkinson, 1979), (tables 3.1.2.1, 3.1.3.1, 3.2.1, 3.2.2, 3.3.2.1, 3.3.2.2), and on occasion were very high (> 200%w/w) (tables 3.1.2.1, 3.1.3.1, 3.2.1 and 3.2.2), However, these high moisture contents had no inhibitory effect on microbial activity (figures 3.2.3-3.2.6), or degradation (figures 3.1.2.2, 3.1.3.4, and 3.2.1) of the wood blocks. Therefore, there was no evidence of waterlogging of the blocks, and the production of anaerobic conditions.

Wood moisture contents of untreated blocks in the CCA soil burial studies were all very similar (tables 3.1.2.1, 3.1.3.1, 3.2.1, 3.2.2). However, the moisture contents of untreated blocks in experimental programme 3 were substantially lower than recorded in the previous studies (tables 3.3.2.1, 3.3.2.2). To determine whether the reduced moisture contents effected the microbial decay of the blocks, the point at which decay of the untreated wood blocks was estimated to have commenced, and the subsequent rate of decay of these blocks were compared (table 4.2.1). The rates of weight loss of the untreated wood blocks in experimental programme 3 were within the range of those recorded previously (table 4.2.1). The only unusual feature of the weight loss of these blocks was that microbial decay of the pine blocks appeared to commence slightly earlier than in the other studies. Since the difference was only a few weeks, and since the 3% weight loss threshold is only an estimate of the point at which significant microbial decay begins, it is reasonable to

conclude the lower moisture contents of the untreated blocks in experimental programme 3 had no effect on the decay of these blocks.

Table 4.2.1 Summary of the estimated time to significant microbial decay of untreated wood blocks and subsequent rate of decay for each wood species.

Experimental programme	Wood species	Leaching	Initiation of decay (weeks)	Rate of decay (%/week)
1 (1)	Pine	Not leached	6.50	1.13
		Leached	6.75	1.14
	Spruce	Not leached	7.75	1.04
	Lime	Not leached	1.50	6.02
1 (2)	Pine	Not leached	7.10	0.62
		Leached	6.75	0.85
	Spruce	Not leached	8.30	0.81
		Leached	7.50	0.82
	Lime	Not leached	0.70	1.91
		Leached	0.70	1.94
2	Pine	Not leached	7.25	0.75
	Spruce	Not leached	5.50	1.08
	Lime	Not leached	2.00	4.43
3	Pine	Not leached	4.80	0.80
	Spruce	Not leached	6.80	0.75
	Lime	Not leached	1.20	3.65

From the moisture content and weight loss results of the four soil burial studies (tables 3.1.2.1, 3.1.3.1, 3.2.1, 3.2.2, 3.3.2.1, 3.3.2.2; figures 3.1.2.2, 3.1.3.4-3.1.3.6, 3.2.1, 3.3.2.1), it appears that microbial decay of the untreated wood blocks began over a wide range of wood moisture contents (between 40 and 187%w/w). Decay then proceeded at very similar rates regardless of the moisture content.

During each study the moisture contents of the decaying blocks increased, with little, or no, change in the moisture contents of the sound blocks. Under aerobic conditions decomposition of wood by microorganisms releases carbon dioxide, water and heat (Boddy, 1983). The breakdown of wood structure during decay also increases the permeability of the wood, allowing an increased absorption of water (Cartwright and Findlay, 1958).

CCA treatment was found to reduce the moisture contents of the buried wood blocks (tables 3.1.2.1, 3.1.3.1, 3.2.1 and 3.2.2); this

phenomenon has been reported previously (Baines, 1982; Murphy, 1982; Gersonde and Kerner, 1984; Gray, 1986; Pizzi and Conradie, 1986) and was attributed to the chromium (VI) component of the preservative (Pizzi and Conradie, 1986 *op cit*).

Baines (1982) suggested that the different moisture contents in untreated and CCA-treated wood make it unwise to relate the performance of treated wood to that of untreated wood, as the organisms responsible for decay in the two woods may consequently not be the same. However, the current work demonstrated substantial differences in the moisture contents of untreated wood did not affect microbial decay (tables 3.1.2.1, 3.1.3.1, 3.2.1, 3.2.2, 3.3.2.1, 3.3.2.2; figures 3.1.2.2, 3.1.3.4, 3.2.1, 3.3.2.1). Therefore, while different microorganisms decay untreated and CCA-treated wood (Nilsson and Henningsson, 1978; Murphy, 1982), this cannot be due solely to the different moisture conditions within the two wood types.

No difference in moisture uptake of ACA-treated and untreated blocks was apparent after soil burial (tables 3.3.2.1 and 3.3.2.2). However, due to the relatively low moisture contents of all blocks in this soil burial study, it would be unwise to consider ACA-treatment of wood blocks to have no effect on moisture uptake on subsequent soil burial.

Ammonia-treatment significantly increased the moisture contents of the lime blocks, suggesting the ammonia imparts a hydrophilic quality to the wood, though the softwood blocks were unaffected (tables 3.3.2.1-3.3.2.3). Ammonia-treatment also substantially increased the rate of weight loss of the lime blocks, though the weight loss of the softwood blocks was less affected (figure 3.3.2.1). Since the decay of wood increases its moisture content (Cartwright and Findlay, 1958; Boddy, 1983), the greater moisture

contents of the ammonia-treated lime blocks could partly be due to their greater weight loss. Furthermore, since ammonia did not affect moisture uptake in the softwood blocks, where decay rates were less affected by ammonia-treatment, it is likely the greater moisture contents of the ammonia-treated lime blocks were wholly due to the greater weight loss of these blocks.

The reduced moisture uptake by CCA-treated pine blocks which had been pre-burial leached was unexpected (figure 3.1.2.1), particularly as the moisture uptake by untreated pine blocks was unaffected by leaching. This pre-burial leaching phenomenon was subsequently found to occur in CCA-treated spruce and lime blocks (figures 3.1.3.2 and 3.1.3.3); again untreated wood blocks were unaffected (figure 3.1.3.1). Furthermore, a less rapid microbial colonisation (figure 3.1.3.7) and hence a delayed onset of decay of leached, 3%w/v CCA-treated lime blocks was also observed (figure 3.1.3.5).

The further decrease in moisture uptake by CCA-treated blocks in soil contact associated with leaching has not been reported previously, to the knowledge of the author. There are a number of possible explanations for the reduction in moisture uptake by leached, CCA-treated blocks. Greaves (1974) found leaching produced a redistribution of preservative metals within the cell walls of CCA-treated specimens, while Drysdale (1983) demonstrated leaching increased the copper and arsenic concentrations in the outer surface of CCA-treated radiata pine stakes. Therefore, redistribution of preservative metals during cold water leaching of the CCA-treated pine, spruce and lime blocks, either on a micro or macro scale, may be responsible for their reduced moisture uptake.

Dahlgren (1975a), while studying the influence of the choice of wood species and preservative composition on the leachability of copper from treated wood, employed a rapid leaching procedure on the

grounds that further reactions of the preservative metals could occur during leaching. Further fixation reactions in leached, CCA-treated wood blocks prior to their emplacement in soil, not occurring in the unleached material, could account for the subsequent difference in moisture uptakes.

Since water was present in all preservative treated wood blocks, it is possible preservative metal redistribution or further fixation reactions could occur during soil burial as well as during leaching. However, as the leached blocks were dried at ambient temperatures immediately before soil burial, it is possible any redistributed preservative metals and/or further reaction products could have been precipitated at this stage.

Pre-burial leaching was found to increase the durability of 3%w/v CCA-treated lime blocks (section 3.1.3.2, figures 3.1.3.5-3.1.3.7). Hill *et al* (1986), included unleached and leached 2%m/m CCA (Tanalith CT106)-treated beech (*Fagus sylvatica*) stakes in their soil-bed test; no major loss of efficacy following leaching was observed. Instances of leached stakes having lower measured weight losses than corresponding unleached blocks were reported (Hill *et al*, 1986 *op cit*), as was found in the current work, though there was no consistent pattern. While moisture contents were measured in the soil bed study of Hill *et al* (1986 *op cit*), they were not reported, consequently it is not known whether leaching had any effect on this parameter in their study.

While the leached, CCA-treated blocks had lower moisture uptakes than the unleached, CCA-treated blocks (figures 3.1.2.1, 3.1.3.2, 3.1.3.3), their moisture contents were still greater than the accepted moisture limits (24-30%w/w, Wilkinson, 1979). Furthermore, despite large differences in the moisture contents of the buried, untreated blocks, comparable rates of decay for each wood species

were observed in the current work. Therefore, the later failure of the leached, 3%w/v CCA-treated lime blocks may be attributed to factors other than the moisture contents of these blocks and/or the factor(s) responsible for the reduction in moisture uptake.

The microbial population of soil adjacent to CCA-treated wood blocks has been demonstrated to have a greater proportion of copper-tolerant wood decay fungi in its population than in soil adjacent to untreated wood blocks (Murphy, 1982). This difference was associated with increased copper contents in the former soil (Murphy, 1982). Furthermore the soft rot decay fungi capable of degrading CCA-treated wood are generally recognised as possessing some metal tolerance (Daniel and Nilsson, 1988). Therefore the increased copper contents of soil adjacent to CCA-treated wood could result in a microbial population more capable of decaying the treated wood than the population surrounding untreated material. An accumulation of copper was measured in soil adjacent to all CCA-treated wood blocks in the current work (figure 3.1.2.8). However, accumulations around around leached, CCA-treated pine blocks were significantly lower than around the equivalent unleached blocks (table 3.1.2.27). Thus, it is probable a difference would be found between soils adjacent to unleached and leached, CCA-treated lime blocks, although this was not investigated. Consequently, there could be a reduced selective pressure for copper-tolerant microorganisms in soil adjacent to leached, CCA-treated wood blocks. This would result in a soil population less able to degrade the treated wood than the microorganisms surrounding unleached material, thus increasing the durability of the leached, CCA-treated wood.

Methods of evaluating wood preservatives are still under review (Hedley and Butcher, 1985). In many tests leaching has been used to accelerate the procedure, as well as to evaluate the impact of

environmental factors. The leaching procedure described in BS 5761:Part 2 (1980) is recommended for use in order that any loss in effectiveness of the test preservative, compared with test specimens which have not undergone any leaching procedure, can be assessed. Leaching procedures similar to BS 5761;Part 2 (1980 *op cit*) are regularly used in studies in the field of wood preservation, though unleached material is not always included in subsequent tests (Bravery, 1968b; Butcher *et al*, 1977; Gray and Dickinson, 1982; Gersonde and Kerner, 1984). In view of the moisture content results reported here it is recommended that unleached blocks are always included with leached blocks in soil burial experiments; currently this is only rarely done.

4.3 Weight loss of the buried wood blocks.

Reasonable reproducibility of the weight losses between the different soil burial studies of the current work were obtained (figures 3.1.2.2, 3.1.3.4-3.1.3.6, 3.2.1, 3.3.2.1). In previous work at this laboratory (Briscoe, 1987; Nayagam, 1987) untreated pine and spruce blocks had average weight losses in the range 12-14% after 12 weeks of soil burial. Untreated lime blocks sacrificed by these workers at the same sampling time had weight losses of between 30 and 65%. Average weight losses of untreated softwood blocks sampled at the 12 week burial time in the current work were slightly lower than those measured by Briscoe (1987 *op cit*)and Nayagam (1987 *op cit*). However, weight losses of the untreated lime blocks in the current work were within the range of those measured previously. The generally good reproducibility of the untreated wood weight losses in the current work indicates the decay potential of the soil utilised was adequate for such tests, and showed no unusual characteristics.

Weight losses of the untreated wood blocks measured in the current work reflect the greater susceptibility to decay by soft rot fungi of the hardwood lime compared with the softwoods, as reported by previously (Savory, 1954a; Duncan, 1960; Smith, 1969a; Behr, 1973). The weight losses of the 3%w/v CCA-treated lime blocks and the 0.25%w/v CCA-treated softwood blocks illustrate Hulme and Butcher's (1977c *op cit*) finding of a 10 to 20-fold difference in the threshold values of CCA in hardwoods and softwoods. The range of data available for the ACA-treated wood blocks was not sufficient for the same conclusion to be drawn. However, the early failure of the 0.14%w/v ACA-treated lime blocks compared with the durability of the 0.07%w/v ACA-treated softwood blocks (figure 3.3.2.1) suggests a similar result would be obtained.

Percentage weight loss is generally found to be proportional to the duration of microbial attack (Cartwright and Findlay, 1958), as was found for most decaying blocks in the current work. However, once the weight loss of the untreated lime blocks reached approximately 55%, there was a reduction in the rate (table 4.2.2). Cellulose and hemicellulose, both of which can be degraded by soft rot fungi, constitute about 75% of hardwoods such as lime (Thomas, 1977). with lignin, which is only slightly degradable, constituting a further 20%. Thus, at a weight loss of 55% it is likely most of the easily accessible cellulose and hemicellulose will have been degraded by the microorganisms, hence the reduction in the rate of weight loss may reflect the increasing difficulty of the microorganisms to utilise the remaining wood substance.

Pre-burial cold water leaching removes all soluble nutrients, such as sugars (R.S. Smith, 1976). Based on the model of wood decay proposed by Smith (1980) a delay in microbial decay of the leached, untreated wood blocks would be predicted, since no soluble nutrients will be released into the soil to annul fungistasis. However, in the current work pre-burial leaching had no effect on the weight losses of the untreated blocks (figures 3.1.2.2, 3.1.3.4; table 3.1.3.5) This is consistent with the importance of wood volatiles, rather than soluble materials, in the decay of wood in soil contact, since wood volatiles were demonstrated to be unaffected by leaching (Mowe, 1983).

Previous work in this laboratory using material from the evaporative surface of dried planks (RSN) demonstrated such specimens had higher decay rates than centre wood samples (Waite and King, 1979; Briscoe, 1987). This could be interpreted as confirming the hypothesis of Smith (1980, *op cit*), and being in conflict with the observations of the current work. However, this hypothesis assumes

that the soluble nutrients, which are redistributed during drying of the green wood, remain soluble after the wood has been dried - first at 40°C and later at 102°C. Nayagam (1987) added soluble sugars and amino acids to centre wood samples and found neither increased the rate of weight loss of the non-preservative treated blocks. Thus, it is possible some of the additional nutrients present in the untreated RSN blocks were not readily water soluble. Therefore, their decay may have been more rapid due to their higher nutrient status, rather than the leaching of soluble nutrients into the soil.

The increase in weight loss as a result of ammonia-treatment observed in spruce, lime and, probably, pine blocks (figure 3.3.2.1; table 3.3.2.6) could be due to readily soluble nitrogen rapidly diffusing from these blocks upon emplacement in soil (figures 3.3.2.2-3.3.2.4). This soluble nitrogen could promote microbial colonisation of the wood. In this regard, Mowe (1983) demonstrated that *Pseudomonas aeruginosa*, a bacterial species known to colonise wood, exhibited a positive chemotactic response to ammonium ions and towards various wood extracts. Although no attempt was made to investigate the effect of added ammonium ions on the chemotactic response, a concentration gradient of ammonium ions and soluble nutrient around the buried, ammonia-treated wood blocks may produce a more rapid bacterial colonisation of ammonia-treated wood blocks. Since bacteria are considered to be the initial colonisers of wood in soil contact (Clubbe and Levy, 1982), an increase in the rate of colonisation by them might lead to an overall increase in the decay rate, as was found in the current work. Microbial activity in adjacent soil was found to be slightly greater around ammonia-treated lime blocks compared with the untreated wood after 2 and 3 weeks of soil burial (figure 3.3.2.14), though no differences were observed for the softwood blocks (figures 3.3.2.12, 3.3.2.13). Dehydrogenase

activity in the outer wood surface of the ammonia-treated blocks was generally higher during the initial stages of decay (figures 3.3.2.6, 3.3.2.8). This is consistent with a more rapid colonisation of the ammonia-treated wood blocks, though there is no evidence these initial colonisers were motile bacteria.

As nitrogen is generally considered to be a limiting factor in microbial decay of wood (Findlay, 1934, Levi and Cowling, 1973; Levy, 1973), the remaining nitrogen in the ammonia-treated wood blocks, even after the loss of readily soluble nitrogen (figures 3.3.2.2-3.3.2.4), could also be responsible for their increased weight loss, assuming at least some of the remaining nitrogen is available to colonising microorganisms. However, the increased rate of weight loss of the ammonia-treated wood (figure 3.3.2.1) cannot be definitely attributed either to the readily soluble nitrogen rapidly lost on soil burial or to the raised nitrogen content of the wood.

A substantial reduction in the decay rate of the 0,14%w/v ACA-treated lime blocks was observed once they had lost about 40% of their weight (figure 3.3.2.1). Before the substantial reduction in the decay rate of the 0.14%w/v ACA-treated lime blocks, their rate of weight loss was greater than the untreated lime blocks, though it was less than the rate for ammonia-treated lime (figure 3.3.2.1). This indicates that in a large portion of the ACA-treated wood decay was promoted by the increased nitrogen content, with only a slight suppression in decay rate as a result of the presence of the preservative metals. Ruddick (1979) observed an increased gross penetration of the wood by ammonia after ACA-treatment. The increase in the initial decay rate of the ACA-treated lime reported here indicates there may also be a better microdistribution of the additional nitrogen than the preservative metals. The reduction in the decay rate of the 0.14w/v ACA-treated lime blocks after they had

lost 40% of their weight may indicate the toxic elements of ACA are fixed to the less readily degraded portion of the treated wood, presumably the lignin and cellulose or hemicellulose associated with lignin. However, ACA has previously been reported to give better penetration of toxic elements than CCA in wood cell walls (Henningsson *et al*, 1980; Greaves and Nilsson, 1982).

The rate of decay of lime blocks was reduced by treatment with a 0.5%w/v CCA solution (figure 3.2.1), as proposed by Smith (1980). However, treatment of the hardwood blocks with a 0.14%w/v ACA solution, having approximately the same copper concentration as the 0.5%w/v CCA solution, produced an increase in the initial decay rate (figure 3.3.2.1). Thus the toxic values of copper were increased by treatment with ACA. This observation was also made by Sundman (1984) for *Pinus radiata* and *Fagus sylvatica* in a fungal cellar test and by Briscoe (1987) for Scots pine, Sitka spruce and lime in a soil burial test, though Hulme and Butcher (1977c) found no difference in the toxic values of copper necessary to prevent soft rot in five species of hardwoods. The increased toxic value of copper in ACA-treated material (figures 3.2.1, 3.3.2.1) may be due to their increased nitrogen contents (figures 3.2.2, 3.3.2.2-3.3.2.4). However, Chou *et al* (1973) suggested the toxicity of CCA lies in the concerted action of all three toxic elements, as even the most resistant fungi are only tolerant to one of the three metals. As there are only two preservative metals in ACA-treated material, and as much of the arsenic is lost during leaching or soil burial (figure 3.3.1.2; table 3.3.2.22), copper is apparently the major toxic metal present in ACA, unlike CCA; this may also account for the increased toxic value of copper measured in the former case.

Butcher (1968) observed increases in the incubation period of a soft rot test increased the toxic values determined. This phenomenon

was also observed in the current work: 3%w/v CCA-treated lime blocks had not failed after 12 weeks of soil burial (figure 3.1.2.2), though after a 36 week study soft rot decay of these blocks was evident (figure 3.1.3.5). Therefore, while 3%w/v CCA-treated softwood blocks and the 5%w/v CCA and 1.41%w/v ACA-treated blocks of all three wood species were resistant to soft rot attack in all studies in the current work (figures 3.1.2.2, 3.1.3.5, 3.1.3.6, 3.2.1, 3.3.2.1), this may not be the case if the studies had been extended beyond 36 weeks.

4.4 Changes in the nitrogen contents of wood blocks during cold water leaching and soil burial studies.

Treating wood with ammonia or ACA solutions increases their nitrogen content (tables 3.3.2.9, 3.2.2.10; Ruddick, 1979; Briscoe, 1987). In this study leaching reduced the initial nitrogen contents of the 1.41%w/v ACA-treated wood blocks by about 60% (figure 3.3.1.3) Similar rapid losses of nitrogen from ammonia and ACA-treated wood blocks were observed during soil burial (figures 3.3.2.2-3.3.2.4). The decrease in the nitrogen as ammonia contents of the ammonia and ACA-treated wood blocks occurred soon after their emplacement in soil (figure 3.3.2.5) and accounted for the majority of the fall in the nitrogen content (table 3.3.2.15). Thus it is evident much of the additional nitrogen retained in the treated wood is present as ammonium ions, as in the original solution. After the rapid decrease in their nitrogen contents, the nitrogen concentrations of buried, 1.41%w/v ACA-treated wood blocks were similar to those in the leached, 1.41%w/v ACA-treated blocks (tables 3.3.1.1, 3.3.2.9, 3.3.2.10). This implies similar processes occurred during leaching and soil burial.

The soluble nitrogen moving into the soil from the ammonia and ACA-treated wood blocks to the adjacent soil must have been far greater than levels diffusing from the untreated wood, since the latter blocks contained negligible quantities of soluble nitrogen (section 3.1.1.5). The increased amounts of soluble nitrogen could stimulate additional microbial activity in soil adjacent to the ammonia and ACA-treated wood, as proposed for wood soluble nutrients (Smith, 1980). However, no general increase in microbial activity in soil adjacent to ammonia and ACA-treated wood blocks was observed (figure 3.3.2.15).

Following the loss of soluble nitrogen from ammonia and ACA-treated wood blocks, the nitrogen contents were still greater than the concentrations in corresponding untreated wood (figures 3.3.2.2-3.3.2.4). Ruddick (1979), also observed increased nitrogen contents in surface sapwood samples of ACA-treated white spruce (*Picea glauca* [Moench] Voss) poles, stored uncovered for two years. In addition to being insoluble in water, the additional nitrogen in the treated wood was unaffected by heating at 102±2 °C, (carried out on all buried wood blocks to determine their dry weight). Furthermore less than half the additional nitrogen was driven off from the wood by steam and 40%w/v sodium hydroxide solution during the nitrogen as ammonia determinations (table 3.3.2.16). However, some of the additional nitrogen retained within the wood appears to be available to colonising microorganisms, since ammonia-treatment increases the decay rate of the wood (table 3.3.2.6), also the use of ACA increases the toxic values of copper compared with CCA (figures 3.2.1, 3.3.2.1; Sundman, 1984; Briscoe, 1987).

Ruddick (1979) found concentration gradients of nitrogen, copper and arsenic concentrations in cross sections of ACA-treated poles; a substantial section of the inner portion of these poles contained little or no preservative metals, though they did have raised nitrogen contents. Ruddick (1979 *op cit*) suggested damage to the well treated outer shell of ACA-treated poles could expose wood with high nitrogen and low preservative contents. Low preservative retentions of ACA, such as 0.14%w/v in lime, render the wood more susceptible to decay than if no preservative treatment was given (figure 3.3.2.1).

Nitrogen concentrations in the ammonia and ACA solutions were similar at about 1.35%w/v (appendix 1). However, the nitrogen contents of the unburied, treated wood blocks and those removed at the first sampling interval were dissimilar and increased in the

order,

ammonia < 0.07/0.14%w/v ACA < 1.41%w/v ACA.

Ruddick (1979 *op cit*) also observed greater amounts of nitrogen in association with higher preservative metal retentions. This implies some of the additional nitrogen is complexed with the preservative metals within the treated wood. Sundman (1984) suggested copper in ACA-treated wood is probably fixed to different wood components as copper hydroxide, or alternatively as $Cu(NH_3)n(OH)_2$, where n=0-3. However, the formation of complexes between ammonia and copper cannot account for all the additional nitrogen retained in the wood, since the nitrogen content at the first sampling interval of the ammonia-treated wood was also greater than untreated wood concentrations (figure 3.3.2.2-3.3.2.4). As ammonia has a greater hydrogen bonding capacity than water (Bariska and Schuerch, 1977), it is possible some of the additional nitrogen present in the ammonia and ACA-treated wood is retained due to the formation of hydrogen or other chemical bonds.

In all the ACA studies the nitrogen contents of the ammonia and ACA-treated lime blocks were greater than the softwood concentrations (table 3.3.2.1; figures 3.3.2.2-3.3.2.4), even when the water soluble nitrogen had been removed. The majority of this difference could be accounted for by the greater nitrogen contents in the untreated lime blocks (table 3.3.2.1; figures 3.3.2.2-3.3.2.4). However, the differences in the nitrogen contents of the untreated lime and softwood blocks does not account for all of the difference in the nitrogen concentrations of the treated material. The greater concentration of nitrogen retained within the ammonia and ACA-treated lime blocks could be due to the different chemical compositions of the softwoods and hardwoods (see section 1.2.4). If the increased level of additional nitrogen retained within the treated lime blocks

is available to colonising microorganisms, the susceptibility to soft rot decay can be expected to increase.

When buried wood blocks were treated with sufficient CCA preservative to prevent decay, small, but significant increases in their nitrogen contents were sometimes observed (figures 3.1.2.3, 3.3.2; tables 3.1.2.7, 3.2.8, 3.2.10). Increases in nitrogen contents of wood in soil contact have been shown to be primarily due to microorganisms (Waite and King, 1980), with the movement of soluble nitrogen from soil into wood generally being negligible (King *et al*, 1976; Waite and King, 1979). Therefore, the increase in the nitrogen contents of the non-decaying CCA-treated blocks indicate a limited microbial colonisation of the wood, even at high preservative concentrations (5%w/v CCA).

Mowe (1983) showed untreated and CCA-treated wood could stimulate hyphal growth towards the wood blocks. It is possible fungal growth was stimulated towards both untreated and CCA-treated blocks, resulting in colonisation, producing the increased wood nitrogen contents observed (figures 3.1.2.3, 3.3.2). In the case of the CCA-treated wood colonising microorganisms were apparently unable to degrade the substrate, thus the increase in wood nitrogen contents were only small (figures 3.1.2.3, 3.3.2). For decaying wood blocks the continuing fungal colonisation of the wood resulted in a continual increase in their nitrogen contents.

The nitrogen contents of the buried, non-decaying, ACA-treated wood blocks did not change during the study (figures 3.3.2.2-3.3.2.4; tables 3.3.2.11). This may indicate ACA-treated wood is less readily colonised by microorganisms than CCA-treated wood. However, this is not in agreement with the weight losses of the 0.14%w/v ACA-treated lime blocks, and the ammonia-treated and untreated wood blocks of all three wood species, where the additional nitrogen present in the two

former cases was found to increase microbial decay (figure 3.3.2.1). It is possible the relatively large nitrogen contents of the 1.41%w/v ACA-treated wood blocks masked small nitrogen increases, though the results do not indicate even small, insignificant increases (figures 3.3.2.2-3.3.2.4).

Generally the nitrogen contents of all untreated and CCA-treated wood blocks increased before significant decay was measured (figures 3.1.2.3, 3.1.3.7, 3.2.2, 3.3.2.2-3.3.2.4). The nitrogen contents of untreated softwood blocks were generally between 0.07-0.12%w/w (tables 3.1.2.6, 3.2.11) (equivalent to a carbon:nitrogen ratio of about 400:1-233:1), before microbial decay occurred, while the nitrogen contents of the untreated lime blocks were in the range 0.138-0.160%w/w (tables 3.1.2.6, 3.2.11) (equivalent to a carbon:nitrogen ratio of approximately 200:1-175:1) when significant weight loss first occurred. Thus it appears microbial decay of the untreated wood was initiated at carbon:nitrogen ratios of about 400:1-175:1. These values are lower than those considered a prerequisite for cellulase activity in the soft rot fungi (Levi and Cowling, 1969; Waite and King, 1979). However, it is the surface of the wood which is decayed by these fungi. Thus, as the dehydrogenase studies on buried wood show (figures 3.2.3-3.2.6, 3.3.2.6-3.3.2.11) the levels of microbial activity in the wood surface were greater than in the remainder of the block, it is possible the nitrogen contents of the decaying blocks were greater at the soil-contact surface than in the inner area of the block. Thus, in the outer wood surface the carbon:nitrogen ratio may be nearer the ratio proposed for cellulase activity in soft rot fungi (Levi and Cowling, 1969; Waite and King, 1979).

Treatment with CCA did not appear to increase the nitrogen requirement of the wood-destroying microorganisms (figures 3.1.2.7,

3.2.2).

There was little, if any, increase in the nitrogen contents of the ammonia-treated wood blocks prior to the onset of decay (figures 3.3.2.2-3.3.2.4). The increased nitrogen contents of the buried, ammonia-treated softwood blocks were apparently suitable for cellulase production by soft rot fungi on the emplacement of the wood in soil. However, microbial decay of these blocks did not immediately commence upon burial (figure 3.3.2.1). This suggests the additional nitrogen present in the ammonia-treated softwood blocks may not all be readily available to the colonising microorganisms.

Although the nitrogen contents at the first sampling interval of ammonia and 0.14%w/v ACA-treated lime blocks were in excess of the 0.2%w/w concentration considered necessary for cellulase production by soft rot fungi (Waite and King, 1979), microbial decay was apparent soon after their emplacement in soil (figures 3.3.2.1-3.3.2.4). However, since decay of the untreated lime blocks was also measured soon after their emplacement in soil (figure 3.3.2.1), it cannot be concluded that the additional nitrogen in the ammonia and 0.14%w/v ACA-treated lime blocks significantly decreased the induction period of microbial decay.

It has been shown the bulk of nitrogen moving into decaying wood blocks is in the form of microorganisms (Waite and King, 1980). Therefore the continual increases in the nitrogen content of the decaying wood blocks (figures 3.1.2.3, 3.1.3.7, 3.2.2, 3.3.2.2-3.3.2.4) represent a continual microbial invasion of the wood. This was previously observed for decay by soft rot fungi, both in pure culture (King and Waite, 1979) and in soil burial (Waite and King, 1980 *op cit*), though this is not a feature of basidiomycete decay (King and Waite, 1979 *op cit*).

As well as delaying the initiation of microbial decay and reducing the decay rate (Smith, 1980), CCA also reduces the rate of microbial colonisation of the buried wood, as evidenced by the nitrogen contents of the decaying untreated, 0.5% and 3%w/v CCA-treated lime blocks (figures 3.1.3.7, 3.2.2). This may also be the case for ACA-treated wood, though the additional nitrogen present complicates comparisons with untreated wood blocks. Furthermore, the only ACA-treated wood blocks to fail in this project (0.14%w/v ACA-treated lime blocks) decayed more rapidly in the first 6 weeks of burial than the untreated blocks. However, on the basis of the nitrogen contents, 0.07%w/v ACA-treated softwood blocks and 1.41%w/v ACA-treated blocks of all three wood species suffered no microbial colonisation during 18 weeks in soil contact, a performance which is equal to or better than those treated with CCA of comparable copper concentrations.

4.5 Losses of preservative metals from CCA and ACA-treated wood blocks and their accumulation in adjacent soil.

A number of workers have noted the lack of correlation between the percentage of preservative elements leached as determined by wood analysis alone and alternatively by analysis of the leach liquors in conjunction with wood analysis (Fahlstrom *et al*, 1967; Norton, 1979; Briscoe, 1987). Fahlstrom *et al* (1967, *op cit*) attributed the difference to the different methods of analysis used to determine the preservative metal contents of the treated wood and the leach liquors. Norton (1979 *op cit*) assessed leach losses using the preservative metal content of matched pairs of unleached and leached, CCA-treated wood blocks, as well as using leach liquor and leached block levels, and suggested a larger number of comparisons would have given a more accurate result in the former case. Briscoe (1987, *op cit*) considered variability in preservative liquid uptake by individual blocks was likely to be responsible for the small leach losses of preservative metals not being detected when comparisons of block analytical results were carried out.

In view of the variability in liquid uptake of individual blocks (table 3.1.2.8) and of other factors, such as selective absorption (Smith and Williams, 1973 ; Henshaw, 1979; King *et al*, 1981b), it is unlikely small preservative metal losses could be measured by the comparison method, even when the same analytical methods are used to determine the preservative metal contents of the treated wood and leach liquors. Therefore, it is not surprising the comparison method gave ambiguous results for preservative metal losses from CCA-treated blocks (table 3.1.1.5), and for copper losses from ACA-treated blocks (table 3.3.1.2), since losses of these metals were generally small (tables 3.1.1.6, 3.3.1.3).

Leach losses of arsenic from ACA-treated blocks, which were far greater than losses measured for any other preservative metal, tended to be underestimated when measured by the comparison method as against the addition method (tables 3.3.1.2, 3.3.1.3). This cannot be explained by any of the factors discussed above and is more likely to be associated with problems in the determination of arsenic content. Forehand *et al* (1967) encountered difficulties in obtaining a stable arsenic hollow cathode lamp for use with the AAS, though the use of an electrodeless discharge lamp improved the reproducibility of their results. In the current work no electrodeless discharge lamp was available, and a hollow cathode lamp was employed in all arsenic determinations, with a certain amount of difficulty being found in obtaining a constant reading. Furthermore, while the standard additions method (section 2.5.4) uses 1 and 2ug/cm^3 for copper and chromium, additions of 10 and 20ug/cm^3 were used in the arsenic determinations. This increases the problems of variability in the readings obtained and of achieving reliable readings with low arsenic concentrations.

The addition method was found to be a more sensitive and accurate technique than the comparison method in the assessment of percentage metal losses after leaching (tables 3.1.1.5, 3.1.1.6, 3.3.1.2, 3.3.1.3). By the addition method leach losses of copper, chromium and arsenic from 3 and 5%w/v CCA-treated wood were generally low , being in the range 0.2-9.5% (table 3.1.1.6), as were losses of copper from 1.41%w/v ACA-treated material (3.8-13.2%, table 3.3.1.3). This is not surprising, since many previous studies have found CCA formulations comparable to the one used throughout the current work are highly fixed within treated wood and consequently are resistant to leaching by water (Fahlstrom *et al*, 1967 *op cit*; Henry and Jeroski, 1967; Dahlgren, 1975; Henshaw, 1979 and Plackett, 1984). Copper in ACA

formulations has also been shown to be leach resistant (Da Costa, 1955; McCarthy and Wilson, 1957; Rak, 1976). Leach losses of copper from 1.41%w/v ACA-treated wood blocks are only slightly greater than those from the 5%w/v CCA-treated wood blocks (tables 3.1.1.6, 3.3.1.3). Since both preservative solutions contained equivalent amounts of copper, it is evident that copper is fixed to the same degree in both ACA and CCA-treated wood.

From previous work (Wilson *et al*, 1955; Da Costa, 1955 *op cit*; McCarthy and Wilson, 1957 *op cit*; Rak, 1976 *op cit*) on leach losses of arsenic from ACA-treated wood large arsenic losses from ACA-treated material can be predicted, probably around 50%. Indeed very high losses of arsenic from 1.41%w/v ACA-treated wood were found, and, in the cases of pine and lime, were well in excess of 50% (figure 3.3.1.2, table 3.3.1.3). Such high losses of arsenic reinforce the view of McCarthy and Wilson (1957 *op cit*), that in a preservative of this type a considerable fraction of the copper forms insoluble complexes which do not contain arsenic. A proportion of these complexes may contain nitrogen in as previously suggested in the current work (section 4.4) and by Sundman (1984).

Preservative metal losses from treated wood during soil burial can only be measured quantitatively by measuring the preservative metal contents of the wood. However, the assessment of metal losses by comparing average levels was shown to produce ambiguous results in the leaching studies (tables 3.1.1.5, 3.1.1.6, 3.3.1.2, 3.3.1.3). Although the determination of the preservative metal contents of soil adjacent to CCA and ACA-treated wood blocks provides additional useful information on metal losses, it is only qualitative.

Generally no losses of preservative metals were found from the preservative metal contents of the CCA-treated wood blocks (figures 3.1.2.4-3.1.2.7, table 3.1.2.15). On two of the occasions on which a

significant decrease in the preservative metal contents of a set of blocks was measured (the copper and arsenic contents of the leached, 5%w/v CCA-treated pine blocks) this appeared to be due to unusually high preservative metal contents in the unburied blocks, rather than to any significant loss of the metals during burial. Thus, the analysis of the preservative metal contents of treated wood during the CCA soil burial studies show metal losses from these blocks were negligible, as they were in the leaching studies (table 3.1.1.6). Furthermore, where preservative metal losses are low, methods other than analysis of the substrate, such as analysis of metal concentrations in the leach liquors or adjacent soil are desirable to build up a reliable picture of metal losses.

Generally no significant decreases were measured in the copper contents of the ACA-treated wood blocks during the soil burial study (table 3.3.2.20). On the two occasions when the calculated percentage loss of copper exceeded 20% (0.07%w/v ACA-treated pine and 0.14%w/v ACA-treated lime) significant losses of the metal were indicated. The 0.14%w/v ACA-treated lime blocks lost about 50% of their weight after 18 weeks of soil burial (figure 3.3.2.1), though these blocks had only lost about 20% of their copper content. This suggests the copper in the ACA-treated wood is predominantly fixed to the undecayed portion of the block, presumably the lignin and cellulose/hemicellulose associated with the lignin (section 4.3).

Losses of arsenic from the 0.14%w/v ACA-treated lime blocks were far greater than those observed for the 1.41%w/v ACA-treated lime blocks during the soil burial study (table 3.3.2.22). It is possible the greater percentage arsenic loss from the less heavily treated blocks occurred as a result of their substantial weight loss (figure 3.3.2.1). However, the soil preservative metal content results indicate (figures 3.3.2.17) most of the arsenic loss from the

0.14%w/v ACA-treated lime blocks occurred during the first few weeks of the soil burial study. This suggests most of the arsenic loss from the 0.14%w/v ACA-treated blocks resulted from poor fixation of the arsenic within these wood blocks. Furthermore, the reduced percentage loss of arsenic from the ACA-treated lime blocks with the increase in preservative concentration suggests that fixation of arsenic is improved with the increase in preservative solution concentration.

The decrease in arsenic contents of the 1.41%w/v ACA-treated wood blocks after 18 weeks soil burial appeared to be less than losses determined after leaching (tables 3.3.1.2, 3.3.2.22). The reduced loss of arsenic from the ACA-treated blocks after soil burial suggests arsenic accumulating in the adjacent soil could have prevented further arsenic loss due to an equilibrium between the wood and soil arsenic concentrations. However, the daily changing of the leach liquor during leaching promoted a greater loss of arsenic, as equilibrium is not achieved under such conditions. Therefore, in this case, cold water leaching apparently exaggerated arsenic losses occurring from 1.41%w/v ACA-treated wood during soil burial. However, the losses measured during the leach study would still be relevant in marine and certain above ground situations.

In most cases the percentage losses of preservative metals CCA-treated wood after leaching decreased in the order,

copper > arsenic > chromium,

for each wood species and treatment (table 3.1.1.6). This order was also obtained for the levels of preservative metals accumulating around the CCA-treated wood blocks during soil burial (figures 3.1.2.8-3.1.2.10). In other studies employing CCA formulations similar to the one used in the current work very low losses, if any, of chromium were found (Dunbar, 1962; Fahlstrom *et al*, 1967; Dahlgren, 1975; Evans, 1978; Henshaw, 1979; Briscoe, 1987). Dahlgren

(1975 *op cit*) found the percentage losses of copper and arsenic were very similar, while Evans (1978 *op cit*) and Henshaw (1979 *op cit*) found arsenic losses were greater than those of copper. On the other hand, studies by Dunbar (1962 *op cit*) and Fahlstrom *et al* (1967 *op cit*) demonstrated greater percentage copper losses than of chromium and arsenic, as was found in the current work. Since the order of decreasing levels of preservative metal losses were the same in the leaching and soil burial studies it is evident the soil has no effect on the relative order in which the three preservative metals are lost from the treated wood. This was also found for ACA-treated material (tables 3.3.1.3; figures 3.3.2.16, 3.3.2.17).

Pre-burial leaching reduced the level of copper accumulating around the treated wood blocks (table 3.1.2.27), though significant increases in copper around the leached, CCA-treated pine blocks did occur (figure 3.1.2.8; table 3.1.2.25). This may indicate burial in soil resulted in additional losses of copper from treated wood blocks. Plackett (1984) demonstrated enhanced leach losses of copper from CCA-treated wood when inorganic salt solutions were used as the leach liquors. The inorganic salts used contained calcium, potassium and magnesium ions, all of which are present at higher concentrations in horticultural soils than in pastoral soils (Plackett, 1984). However, soil employed in the current work was pastoral soil (see section 2.3.1). Therefore, it would not be expected to contain high concentrations of calcium, potassium and magnesium ions. Since losses of copper from the CCA-treated pine blocks had not ceased on the final day of the leaching study (figures 3.1.1.1, 3.1.1.2) the copper accumulating in soil adjacent to the leached, CCA-treated pine blocks may reflect either a continuing copper loss, which would be expected in a prolonged leaching study, or an increased loss, due to additional factors present in the soil solution.

In this study losses of preservative metals, both during leaching and soil burial, were generally greater from preservative-treated lime blocks than they were from the treated softwoods (tables 3.1.1.6, 3.3.1.3, 3.3.2.20, 3.3.2.22; figures 3.1.2.22-3.1.2.24, 3.3.2.16. 3.3.2.17). Although this is in agreement with the leach studies of Becker and Buchmann (1966), other leaching investigations have found no differences in preservative metal losses from hardwood and softwood species (Henshaw, 1979; Briscoe, 1987). Since hardwood and softwood blocks took up approximately the same volume of preservative solution during treatment (table 3.1.2.8), the difference in preservative metal levels in soil adjacent to preservative treated hardwood and softwood blocks (figures 3.1.2.22-3.1.2.24, 3.3.2.16, 3.3.2.17) cannot be attributed to a greater initial preservative loading in the lime blocks. Rather, it may indicate an incomplete fixation of the CCA within the hardwood blocks. Pizzi (1983) proposed that in most CCA-treated material the majority of the preservative metals are fixed to the lignin rather than to the holocellulose. Since lime contains less lignin than the softwoods (Cote, 1977; Findlay, 1985a), the preservative metals may be incompletely fixed within the treated lime blocks, leading to greater metal losses.

The increased preservative metal concentrations in soil adjacent to the treated wood blocks (figures 3.1.2.8-3.1.2.10, 3.3.2.16, 3.3.2.17) may have had two possible effects, serving either to delay or conversely to promote the microbial colonisation and decay of the preservative treated wood blocks. Smith (1980) suggested the diffusion of sub-lethal amounts of preservative into the adjacent soil could delay or prevent fungal spore germination. Thus, the greater preservative metal concentrations in soil adjacent to the treated hardwood (figures 3.1.2.22-3.1.2.24, 3.3.2.16, 3.3.2.17)

could tend to delay fungal colonisation of these blocks in comparison with the CCA and ACA-treated softwoods. Murphy (1982) demonstrated copper accumulations in soil adjacent to CCA-treated wood; this was associated with a qualitative change in the microbial population adjacent to these blocks when compared to the population in soil adjacent to untreated wood. A far greater proportion of copper tolerant fungi were isolated from the former population. Therefore, the greater accumulations of preservative metals in soil adjacent to treated lime blocks might serve to select a microbial population which is more tolerant to the preservative components than the population in soil adjacent to treated softwood blocks, thus increasing the relative decay hazard for the treated lime blocks. The difference between the soft rot decay susceptibility of the hardwoods and softwoods would thus be further increased by preservative treatment, since the treated hardwoods will be exposed to greater decay hazard than the treated softwoods.

Since the hardwoods and softwoods have different soft rot decay susceptibilities, it is impossible to conclude from the work carried out in this project which is the more plausible explanation. However, on the basis of the dehydrogenase activities in soil adjacent to the preservative treated wood blocks, there was generally no evidence of any sterilising or inhibitory effect on the microbial population (figures 3.2.7-3.2.10, 3.3.2.12-3.3.2.15). However, the peak in dehydrogenase activity observed in all soil samples after approximately 3 weeks was always lower for soil adjacent to preservative treated wood blocks than for soil around untreated wood blocks. This suggests the rapid accumulation of preservative metals around treated wood inhibited the germination of some of the soil microbial population, though a proportion is apparently still capable of germination, despite the preservative metals present. This tends

to favour the proposed decay model of Murphy (1982).

Although in the current work a definite conclusion cannot be reached as to the effects of preservative metals leaching from treated into the adjacent soil, the results obtained are evidently in agreement with the suggestions of Murphy (1982), but not the proposals of Smith (1980).

4.6 Dehydrogenase activity in the outer and inner wood of buried wood blocks.

In this discussion wood dehydrogenase activity will be considered in relation to weight loss and nitrogen contents of the wood blocks during the soil burial studies. However, dehydrogenase activity measurements only give an indication of the microbial activity at the time of sampling, while weight loss and increases in wood nitrogen contents are the result of cumulative microbial activity.

The dehydrogenase assay was selected as a suitable technique to measure levels of microbial activity in untreated and treated wood blocks, and thus to determine the effects of both preservative and ammonia treatment on the activity levels (section 1.5.2). This assay has been used previously in this laboratory to determine microbial activity in samples of buried, 0.5%w/v CCA-treated lime blocks (Mowe, 1983), however comparisons of the current type were not attempted. The outer wood surface, in direct soil contact, and the inner portion of the wood were assayed separately, measurements not previously attempted.

TTC was reduced to TTF in decaying wood blocks (figures 3.2.1, 3.2.3-3.2.6, 3.3.2.1, 3.3.2.6-3.3.2.11). However, activity was not measured in the non-decaying wood blocks. Furthermore, the greatest levels of dehydrogenase activity were generally obtained for wood blocks suffering the greatest rates of decay. Thus, while the TTC assay of dehydrogenase activity does not give absolute levels of microbial respiration (Howard, 1972; Benefield *et al*, 1977), it appears adequate for determining the effect of wood species and treatment on the microbial activity within buried wood blocks.

A more efficient solvent extraction was employed in the wood dehydrogenase assays of experimental programme 3 than in experimental

programme 2 (section 2.4.2), thus the results obtained in the two soil burial studies cannot be directly compared. However, the patterns of dehydrogenase activity levels can be compared.

A comparison of the dehydrogenase activities in untreated and ammonia-treated pine and lime blocks show a more rapid colonisation of the ammonia-treated blocks of both wood species (figures 3.3.2.6, 3.3.2.8, 3.3.2.9, 3.3.2.11). The extent of these differences corresponded with the degree of difference in the weight losses of the untreated and ammonia-treated blocks of each wood species (figure 3.3.2.1). The increased rate of microbial colonisation of the ammonia-treated wood, as evidenced by the greater levels of microbial activity, may be due to a nitrogen concentration gradient in the soil surrounding the buried wood blocks, though this is not consistent with the soil dehydrogenase levels (figure 3.3.2.15). A more plausible explanation is that the additional nitrogen present in the ammonia-treated wood increased the suitability of the wood as a nutrient source for the soil microbial population.

The differences in dehydrogenase activity in the untreated and ammonia-treated pine and lime blocks were not observed in the spruce blocks (figures 3.3.2.7, 3.3.2.10). Levels of dehydrogenase activity in the outer surface and inner wood of untreated and ammonia-treated spruce blocks were consistently lower than those of the comparable pine blocks in both studies (figures 3.2.3, 3.2.5, 3.3.2.8, 3.3.2.9, 3.3.2.11, 3.3.2.12), though their weight losses were not substantially different (figures 3.2.1, 3.3.2.1). There is no evidence to suggest the spruce wood chemically binds the TTF, since recoveries of added TTF from untreated spruce were the same as those from untreated pine and lime samples (section 3.2.8). This suggests the refractory nature of spruce (Wilkinson, 1979) prevented adequate penetration of the TTC solution and/or the methanol into the assayed wood

samples. However, if this is the case, it implies the anatomical features responsible for spruce's refractory nature were not greatly effected by the microbial decay.

Heavy metals present in the CCA and ACA-treated wood reduced the recovery of added TTF (table 3.2.18). Buried material was used in all tests carried out, thus unfixed preservative could not be responsible for the reduction, and, in the case of ACA-treated wood, the amount of arsenic available to interact with the added TTF would be very small. Furthermore, as both CCA and ACA-treated wood reduced the recovery of TTF, it seems most likely the copper present in the preservative treated wood is associated with the reduced TTF recovery. Despite the reduction in recovery of added TTF from preservative treated wood, activity was always measured in the decaying, CCA and ACA treated wood blocks (figures 3.2.4, 3.2.6, 3.3.2.8, 3.3.2.11).

The reduced recovery of added TTF from preservative treated wood eliminates comparisons of microbial activity in the untreated and preservative treated blocks. However, it is still possible to compare patterns of dehydrogenase activities in CCA and ACA-treated wood blocks containing approximately equal quantities of copper. Generally no microbial activity was measured in the preservative treated wood blocks. This was not surprising, since these blocks did not usually suffer any significant microbial decay (figures 3.2.1, 3.3.2.1), though in some cases small but significant nitrogen increases, considered to indicate a limited microbial colonisation (see section 4.4), were measured. Thus, either the small amount of TTC reduced to TTF by any microorganisms present in the blocks was not recovered, or any microbes colonising these blocks were not metabolising.

Dehydrogenase activity in the outer and inner wood samples of the 0.14%w/v ACA-treated lime blocks exhibited a similar pattern to the

activity levels measured in the untreated lime blocks during the same study (figures 3.3.2.8, 3.3.2.11). However, the pattern of activity levels in the 0.5%w/v CCA-treated lime blocks was more like those of the untreated softwood blocks than the equivalent lime blocks (figures 3.2.3-3.2.6). This suggests the low CCA concentration reduced the level of microbial activity within the wood blocks, as was indicated by their reduced decay rate (figure 3.2.1). Since the patterns of dehydrogenase activity in 0.14%w/v ACA-treated lime and untreated lime were similar, and since activity in the former group was not the same as in the CCA-treated wood, there is no evidence to suggest the low concentration of ACA reduced levels of microbial activity. Furthermore, the low concentration of ACA had no apparent inhibitory effect on the degradation of the lime blocks until a large percentage of the wood had been degraded (figure 3.3.2.1).

Microbial activity was measured in untreated and ammonia-treated softwood blocks prior to decay (figures 3.2.1, 3.2.3, 3.2.5, 3.3.2.1, 3.3.2.6, 3.3.2.7, 3.3.2.9, 3.3.2.10); in the untreated wood this was associated with increased wood nitrogen contents (figures 3.2.2, 3.3.2.2, 3.3.2.3). This supports the view (Waite and King, 1980) that increases in the nitrogen contents of the buried wood are predominantly microbial in origin.

In all wood blocks in which decay was observed, dehydrogenase activity was always present. Without exception the average levels of enzymic activity in the outer wood surface were greater than those measured in the inner wood (figures 3.2.3-3.2.6, 3.3.2.6-3.3.2.11), reflecting the surface nature of soft rot decay even in blocks which are only 5mm thick. Nitrogen increases measured in the wood blocks placed in soil contact are primarily due to the microbial biomass moving into the wood (Waite and King, 1980). This implies a

decreasing gradient of nitrogen concentration towards the block centre. However, this aspect was not investigated further in the current work.

The rate of microbial decay of the buried wood blocks generally remained approximately constant (figures 3.2.1, 3.3.2.1). However, microbial activity in the same wood blocks did not remain constant (figures 3.2.3-3.2.6, 3.3.2.6-3.3.2.11). This may indicate a series of changes in the microorganisms involved in the degradation of the wood. Alternatively, the metabolism of the microorganisms decaying the wood may be changing with time.

Far greater levels of dehydrogenase activity were measured in the buried wood blocks, particularly in the outer wood surface, than in the adjacent soil. (figures 3.2.3-3.2.9, 3.3.2.6-3.3.2.14). This may reflect the greater amount of organic matter being decomposed within the wood blocks than within the adjacent soil. However, some workers (Benefield *et al*, 1977) believe TTC may not be a very efficient hydrogen/electron acceptor, only becoming active when other acceptors which may be present in soil are exhausted. Such alternative electron acceptors may not be present to the same extent in decaying wood blocks, which would lead to a greater reduction of TTC to TTF in the wood.

The wood dehydrogenase assay results demonstrate the measurement of microbial activity can provide useful additional information on the utilisation by microorganisms of wood in soil contact. It is not suggested that microbial activity in wood should be the sole parameter measured in a soil burial study, but rather it should be employed in conjunction with other appropriate measurements, such as wood weight losses and nitrogen contents. In view of the interference of waterborne wood preservatives in the recovery of TTF, it is

apparent the TTC dehydrogenase assay cannot be recommended for the assessment of microbial activity in buried, preservative treated wood.

4.7 Dehydrogenase activity in soil adjacent to buried wood blocks.

Two different solvent extraction regimes (section 2.4.2) were used in experimental programmes 2 and 3; the more efficient extraction for the latter programme. A comparison of the dehydrogenase activities in the background (control) soil samples, show the greater levels of activity were measured using the second regime (figures 3.2.7-3.2.10, 3.3.2.12-3.3.2.15), consequently direct comparisons of soil activities are not possible. However, the general patterns of changes in soil microbial activity can be compared.

A feature of all soil samples was an initial flush in microbial activity, usually after about 3 weeks (figures 3.2.7-3.2.10, 3.3.2.12-3.3.2.15). The magnitude of this flush was influenced by the wood species and the preservative treatment, though increased activity was also observed in the background soil. When an air-dried soil is re-moistened there is a flush of microbial activity (Griffiths and Birch, 1961; Powlson and Jenkinson, 1976; Lund and Goksoyr, 1980). This has been attributed to the decomposition of microorganisms killed during air-drying by those which survived (Powlson and Jenkinson, 1976 *op cit*; Lund and Goksoyr, 1980 *op cit*). Some mineralisation of humic substances in the soil is also thought to occur (Lund and Goksoyr, 1980 *op cit*). Since the flushes of dehydrogenase activity (figures 3.2.7-3.2.10, 3.3.2.12-3.3.2.15) and respiration in the soil microbial population (Griffiths and Birch, 1961 *op cit*, Powlson and Jenkinson, 1976 *op cit*, Lund and Goksoyr, 1980 *op cit*) are similar, it is possible the initial peak in activity may be partially due to the increase in decomposable organic matter in the soil. In the background soil the increase in organic matter as a result of air-drying probably accounts for most of the flush.

Bravery (1968a) demonstrated air-drying the soil did not reduce

the wood decaying potential of the natural soil microflora, and may even have enhanced it. He suggested the microorganisms present in fresh soil would be induced to sporulate with the onset of unfavourable conditions as the soil dried out, thus effectively increasing the number of propagules in the drying soil. The flush of microbial activity observed in the current work indicates the enhanced decay potential observed by Bravery (1968a) may also be due to the increased activity of microorganisms capable of decomposing organic matter.

The initial flush of activity was greater in soil adjacent to all lime blocks than in any other soil samples (figures 3.2.10, 3.3.2.15). This difference is not due to the different moisture contents of the soil into which the lime and softwood blocks were placed, since background moisture activities at the two water holding capacities were generally similar.

Soluble nutrients from untreated wood blocks would be expected to diffuse rapidly into adjacent soil, in the same manner as preservative metals (figures 3.1.2.8-3.1.2.10, 3.3.2.18, 3.3.2.19), inducing some microbial germination (Smith, 1980); this may account for the increased flush of dehydrogenase activity around the wood blocks. However, the soluble nitrogen (section 4.3; Nayagam, 1987) and carbohydrate (Nayagam, 1987 *op cit*) contents of the wood are known to be low. Furthermore, the soluble nutrient concentrations in the sub-surface lime samples were only slightly greater than those in the softwoods (Nayagam, 1987 *op cit*). Therefore, while some of the additional flush in activity around the wood blocks may be attributed to soluble nutrients diffusing into the soil, these cannot account for the difference in the initial activity peaks associated with the different wood species.

Mowe (1983), in pure culture agar experiments, observed the

orientation of fungal mycelia by wood volatiles towards unleached and leached wood blocks and suggested volatiles may also stimulate fungal growth. Thus, some of the increased flush in microbial activity in soil adjacent to the blocks may be due to the increase in the fungal population by wood volatiles emanating into the soil. However, Mowe (1983) found no difference in the response of *Chaetomium globosum* towards volatiles of lime and pine. In contrast, Hardie (1979) found lime sapwood possessed a volatile, leachable substance capable of inhibiting the germination of ascospores of *C.globosum*, though no comparable inhibitor was found in Scots pine sapwood. Thus, while wood volatiles may be responsible for microbial germination and colonisation of wood in soil contact, there is no evidence to suggest such volatiles account for the very high levels of activity in soil adjacent to the lime blocks. By necessity the wood volatile studies discussed were carried out using pure culture agar tests, thus it is conceivable the volatile effect is very different in soil, where a varied microbial population is involved.

Microbial decay of the untreated lime blocks began earlier in all soil burials than decay of the softwoods (figures 3.1.2.2, 3.1.3.4, 3.2.1, 3.3.2.1); this may account for some of the additional flush measured in soil adjacent to the lime blocks. King *et al* (1980a) postulated the maintenance of biotic connections between wood and soil during soft rot decay. Therefore, microbial activity in decaying blocks would be reflected in increased activity in adjacent soil. However, the flush in activities around the non-decaying, preservative-treated lime blocks were still greater than obtained for the untreated softwoods. Therefore, increased soil enzymic activity around untreated lime compared with the untreated softwood blocks cannot be attributed solely to microbial colonisation and decay of the hardwood.

No single factor, such as soluble nutrients, volatiles or microbial decay, can apparently account for the greater levels of microbial activity measured in soil adjacent to the lime blocks. The greater levels of microbial activity measured in soil adjacent to the lime blocks may be associated with the rapid colonisation and decay observed in all but the heavily treated lime blocks (figures 3.1.2.2, 3.1.2.3, 3.2.1, 3.2.2, 3.3.2.1-3.3.2.4). This may have implications as regards the greater soft rot decay susceptibility of the hardwoods.

There was a rapid loss of ammonium ions from ammonia and ACA-treated wood on their emplacement in soil (figures 3.3.2.2-3.3.2.5). Ko *et al* (1974, cited in Griffin *et al*, 1975), suggested ammonia as a volatile fungistatic agent in highly alkaline soils. However, it is unlikely the nitrogen diffusing from the ammonia treated wood inhibits germination of a portion of the microbial population, since the majority of the ammonia present in the wood on soil burial will not be volatile at 25°C (the soil temperature), but rather will diffuse from the blocks in solution, as observed in the ACA leaching study (figure 3.3.1.3). Furthermore, the average pH of the soil in this study was 6.2 (appendix 2), which is classified as slightly acid to neutral (BS 3882, 1965). At such a soil pH it is likely the ammonia entering the adjacent soil undergoes nitrification to form nitrate (Brock, 1979), or alternatively will be adsorbed by negatively charged clay minerals (Brock, 1979 *op cit*). Whatever the fate of the ammonium ions diffusing from the ammonia and ACA-treated wood, they had no apparent effect on the flush of dehydrogenase in the adjacent soil (figures 3.2.7-3.2.10, 3.3.2.12-3.3.2.15).

The flush of activity was generally less in soil adjacent to the preservative-treated wood blocks than levels around the untreated

wood (figures 3.2.10, 3.3.2.15). Since neither ACA nor CCA are volatile preservatives the depression in microbial activity is likely to result from preservative metals diffusing rapidly into the surrounding soil (figures 3.1.2.8-3.1.2.10, 3.3.2.16, 3.3.2.17). Such a depression of enzymic activity has previously been demonstrated in heavy metal contaminated soil (Ruhling and Tyler, 1973; Reddy *et al*, 1987). However, the initial peaks in activity were similar for both CCA treating concentrations for each wood species (figure 3.2.10), despite their probable different soil metal accumulations (based on figures 3.3.2.16 and 3.3.2.17). This suggests that, while the activity of a proportion of the microbial population was depressed by relatively small increases in the preservative metal concentrations around the 0.25 and 0.5%w/v CCA-treated wood blocks, it was not increased by the greater accumulations around the 5%w/v CCA-treated wood blocks. This may indicate only some of the preservative metals accumulating in soil around the buried wood blocks affect the soil microflora.

The flush of microbial activity around the 1.41%w/v ACA-treated wood blocks was significantly lower than those of the less heavily ACA-treated blocks (figure 3.3.2.15). The copper contents of soil adjacent to the 1.41%w/v ACA-treated wood blocks were similar to those of the 5%w/v CCA-treated blocks (figures 3.1.2.8, 3.3.2.16). However, while a decrease in activity around CCA-treated wood occurred, this was not affected by the wood, and consequently, soil preservative metal concentration (figure 3.2.10). The arsenic concentrations in soil adjacent to the 1.41%w/v ACA-treated wood blocks were far greater than the levels around any other wood blocks (figures 3.1.2.10, 3.3.2.17). It appears the additional arsenic concentration around the 1.41%w/v ACA-treated blocks has reduced the flush of activity to levels substantially below those of the other

ACA-treated wood blocks.

A difference between the burst in respiratory activity observed (Griffiths and Birch, 1961 *op cit*; Powlson and Jenkinson, 1976 *op cit*; Lund and Goksoyr, 1980 *op cit*) and the peak of dehydrogenase activity (figures 3.2.7-3.2.10, 3.3.2.12-3.3.2.15) is the time taken before the flush is measured; the former took place within a few days of re-wetting the soil. Lund and Goksoyr (1980 *op cit*) observed an increase in plate-counted bacteria which corresponded with the respiratory "burst"; fungal and microscopically counted bacteria continued to increase in numbers for about a further 10 days. Therefore, the time taken for the flushes in respiratory and dehydrogenase activities to be measured after the soil is re-wetted may indicate the two techniques measure activity in different sections of the soil population.

Activity in soil adjacent to all decaying wood blocks was significantly greater than levels in soil adjacent to non-decaying blocks and in background soil in the remainder of the studies (figures 3.2.10, 3.3.2.15). Activity was always greatest around the decaying hardwood blocks, which also had the greatest decay rates (figures 3.2.1, 3.3.2.1). Since a biotic connection is thought to be maintained between wood and soil during soft rot decay King *et al* (1980a), it is not surprising the soil activity remained high around decaying blocks and that the level was related to the decay rate of the wood. However, while the decay rates of the wood blocks generally remained constant (figures 3.2.1, 3.3.2.1), the microbial activities in adjacent soil adjacent tended to fluctuate (figures 3.2.10, 3.3.2.15). Activity around the softwood blocks generally increased in the latter weeks of both studies, while activities around the lime blocks decreased. Fluctuations in activity within the buried wood were also observed (see section 4.6). The changing levels of enzymic

activity associated with the constant decay rates may indicate a succession of different microbial groups or species is occurring in the wood and the adjacent soil. Alternatively it may reflect changes in the metabolism of the microbial population during the decay process.

Activity in soil adjacent to the 0.14%w/v ACA-treated lime blocks was lower during the first 6 weeks of the study than activity around the untreated lime blocks (figure 3.3.2.15), despite the lower decay rate of the latter group of wood blocks in this period. This suggests a difference in the composition of the microbial population in soil adjacent to wood as a result of preservative treatment (Murphy, 1982).

Generally dehydrogenase activities in soil adjacent to untreated spruce blocks were lower than levels adjacent to the untreated pine blocks. While on occasion decay of the spruce blocks was also lower (figure 3.3.2.1), this was not always the case (figure 3.2.1). Thus, untreated pine blocks appear to stimulate somewhat greater activity in adjacent soil than untreated spruce blocks, though this is not necessarily related to the microbial degradation of the wood blocks themselves. Instead it may be connected with some other feature of the composition of the two softwoods.

Following the early flush in dehydrogenase activity in soil around the 5%w/v CCA and 1.41%w/v ACA-treated wood blocks, activity returned to background levels. Therefore, there was no sterilisation of the microbial population due to the preservative metals which have diffused into the surrounding soil (figures 3.1.2.8-3.1.2.10, 3.3.2.16, 3.3.2.17). It appears that where preservative levels are sufficient to prevent utilisation of the substrate, the microbial population will return to its former levels of metabolic activity. However, the microorganisms present within the background soil and

soil adjacent to non-decaying, preservative-treated wood may be different with regard to their preservative metal tolerance (Murphy, 1982).

Average levels of activity around the heavily treated spruce blocks were lower than those of the background soil in the latter half of the study (figures 3.2.10, 3.3.2.15). This suggests an inhibition of microbial activity in soil around these blocks, though this was not apparent in soil around the pine and lime blocks. As decaying spruce wood blocks had lower levels of activity in their adjacent soil than the pine blocks (figures 3.2.10, 3.3.2.15), the reduction in soil dehydrogenase activities around 5%w/v CCA and 1.41%w/v ACA-treated spruce may be related to the wood species, rather than solely to the preservative treatment.

Microbial activity in soil adjacent to 0.25%w/v CCA and 0.07%w/v ACA-treated wood blocks was generally greater than the background levels (figures 3.2.10, 3.3.2.15), though negligible decay of these blocks occurred during the studies (figures 3.2.1, 3.3.2.1). In view of the relationship between microorganisms in the adjacent soil and the colonised wood (King *et al*, 1980a), the increased microbial activities around these blocks may indicate a low level of microbial colonisation occurring. However, microbial activity was not generally measured in these wood blocks and no continual increase in their nitrogen contents was observed (figures 3.2.2, 3.2.3, 3.2.5, 3.3.2.2-3.3.2.4, 3.3.2.6, 3.3.2.7, 3.3.2.9, 3.3.2.10), which suggests microbial colonisation was not occurring.

CHAPTER 5

CONCLUSIONS

CONCLUSIONS

From the results it is concluded that losses of preservative metals from wood treated with CCA type 2 (BS 4072: 1974) were negligible (< 10%) during cold water (20±2°C) leaching and soil burial. Leach losses of preservative metals from the CCA-treated wood and accumulations in adjacent soil decreased in the order, copper > arsenic > chromium. Burial in soil had no effect on the magnitude of preservative metal losses compared with leaching. Copper was equally leach resistant in wood blocks treated with solutions of CCA and ACA containing equivalent amounts of copper. However, leach losses of arsenic from ACA-treated wood were substantial (> 60%), as predicted. There was no difference in the copper losses from 1.41%w/v ACA-treated wood blocks after leaching and soil burial. However, losses of arsenic from ACA-treated wood were lower after burial in soil than after leaching; this is thought to be due to variations in the equilibria of the wood block environment during the two different procedures.

The air-drying and re-wetting of the soil during the soil burial procedure produced an initial flush of microbial activity, which is attributed to the decomposition of organic matter, the concentrations of which were increased by the air-drying. The initial flush was increased around all wood blocks, particularly around lime blocks. The increased flush in activity may result from soluble nutrients and/or volatiles diffusing from the wood into the adjacent soil. The greater microbial activity around the hardwood blocks was associated with more rapid microbial colonisation and degradation of these wood blocks. Consequently, the greater microbial activity around the hardwood blocks may have implications with regard to the greater soft rot susceptibility of these blocks. However, the explanation for the

large flush around the hardwood blocks is unclear. The flush of microbial activity was reduced by preservative treatment of the wood, though the reduction was independent of the CCA treating concentration, indicating not all of the copper accumulating in the adjacent soil is available to affect the microflora. Soil arsenic concentrations were greatest around the 1.41%w/v ACA-treated wood blocks and was associated with a further decrease in the flush of enzymic activity. This may indicate some fungitoxic activity of the arsenic.

There was no evidence of sterilisation of soil adjacent to any preservative treated wood blocks, and generally microbial activity was not reduced below background levels. Preservative treated and untreated spruce blocks appeared to limit the activity levels in adjacent soil and within wood blocks compared with pine, though in all other respects the performance of the two softwoods were similar. Consequently the reasons for the inhibition of the microbial population in and around the spruce blocks are unclear.

Reduced recoveries of added TTF from preservative treated wood were considered to be due to TTF complexing with the copper. Consequently the effect of the preservatives on microbial activity in buried wood blocks could not be fully determined from the wood dehydrogenase activities. However, it can be concluded that low levels of CCA reduce the rate of microbial colonisation and utilisation of the treated wood to a greater extent than ACA at similar copper concentrations. The increased toxic values of copper in the ACA-treated wood are thought to be a consequence of the increased nitrogen content of the treated wood, and/or the reduced total preservative metal concentration within the wood.

Treatment of wood blocks with ammonia or ACA solutions increased their nitrogen contents. Although, some of this nitrogen was water

soluble, its rapid diffusion into adjacent soil did not affect microbial activity in this area. A further portion of the additional nitrogen in the treated wood could not be removed by water, heat (102°C) or treatment with steam and 40% sodium hydroxide solution. It is considered possible some of the additional nitrogen in the ammonia and ACA-treated wood is retained due to the formation of hydrogen or other chemical bonds. In the ACA-treated wood some of the remaining nitrogen is thought to have been present in association with copper as $Cu(NH_3)n(OH)_2$, where n=0-3. The increased nitrogen contents of the ammonia and ACA-treated wood blocks was associated with increased microbial colonisation and degradation, along with the increase in the copper toxic value when compared with CCA-treated wood. Thus it is considered that some of the nitrogen retained in the ammonia and ACA-treated wood is available to colonising microorganisms.

Substantial increases in the nitrogen contents of the decaying wood blocks were observed. Increases in nitrogen contents have previously been considered to be predominately microbial in origin and the results herein show dehydrogenase activity is associated with increases in nitrogen contents immediately prior to and during microbial decay. The continual increases in wood nitrogen content are a feature of soft rot decay. Microbial activity was greater in the outer surfaces of the buried wood blocks than in the remainder of the wood, demonstrating the surface nature of soft rot decay. Generally the decay rate of wood blocks remained approximately constant, though the microbial activity in the wood and the adjacent soil tended to fluctuate. This is thought to reflect either a change in the composition of the microbial population in the wood and soil over this period, or alternatively changes in the metabolism of the microflora.

Pre-burial leaching of CCA-treated wood blocks reduced their

subsequent moisture uptake and increased their durability during soil burial. This unexpected phenomenon may be due to a macro or micro re-distribution of the preservative metals, or to the production of different fixation products as a result of leaching. Similar rates of decay were observed for untreated wood blocks at a variety of different moisture uptakes. Consequently, it is considered the increased durability of the leached, CCA-treated wood may not be due solely to their lower moisture contents. Rather, it may be a result of the causal factor in their reduced moisture levels or alternatively could be a consequence of a reduced selective pressure for copper-tolerant microorganisms in the adjacent soil microflora.

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APPENDICES

APPENDIX 1. Details of preservative solutions used.

Table 1. pH, densities and nitrogen contents of preservative solutions used (mean value of three replicate samples).

Preservative solution	Salt concentration (%w/v)	pH	Density (w/v)	Nitrogen content (%w/w)
CCA	0.25	2.67±0.010	1.002±0.000	M/N
	0.5	2.41±0.014	1.004±0.000	M/N
	3.0	2.13±0.003	1.026±0.001	M/N
	5.0	2.04±0.005	1.044±0.001	M/N
ACA	Ammonia solution	11.48±0.005	M/N	1.35±0.011
	0.07	11.21±0.005	0.994±0.0001	1.37±0.013
	0.14	11.08±0.003	0.994±0.0001	1.36±0.003
	1.41	10.12±0.003	1.008±0.0001	1.31±0.000

Table 2. Preservative metal contents (ug cm^{-3}) of solutions used (mean value of three replicate samples).

Preservative solution	Salt concentration (%w/v)	Element concentration ($ugcm^{-3}$)		
		Copper	Chromium	Arsenic
CCA	0.25	216.0±10.0	365.2±23.0	280.2*
	0.5	417.9±28.6	752.2±22.9	560.4*
	3.0	2578.1±65.3	4878.6±431.5	3362.0*
	5.0	4270.6±260.8	8090.0±403.4	5604.0*
ACA	Ammonia solution	M/N	M/N	M/N
	0.07	200.7±24.4	M/N	206.3±22.2
	0.14	420.0±33.0	M/N	367.6±40.0
	1.41	4573.9±157.7	M/N	3311.6±256.7

Key.

M/N Measurement not carried out

* At the time of impregnation arsenic analysis was unavailable, therefore the arsenic concentration was calculated using the weight of arsenic pentoxide in each preservative solution.

Appendix 2.

Details of soil used in the burial studies.

pH of the soil.

Duplicate readings were taken on three samples of soil.

pH of the soil used = 6.225 ± 0.035

According to BS 3882 (1965), the soil used in these studies would be classified as slightly acid to neutral (pH 6.0-7.0).

Moisture content of soil on setting up the studies and at 100% of its water holding capacity. (Mean values of three replicate samples).

Experimental programme	Storage bin number	Soil moisture content (%w/w)	
		On setting up the experiment	At 100% water holding capacity
1 (1)	1	18.58±0.32	24.48±1.12
	2	19.49±0.26	24.55±0.94
1 (2)	1	10.80±0.24	21.35±0.76
	2	12.54±0.26	22.56±1.01
	3	16.46±0.23	22.66±0.24
2	1	15.72±0.45	24.74±1.58
	2	14.70±0.26	23.67±0.64
3	1	9.66±0.12	22.40±0.34
	2	9.01±0.08	21.51±0.65
	3	8.82±0.19	20.68±0.52
	4	9.26±0.19	20.22±0.45